



US009272022B2

(12) **United States Patent**
Chang et al.

(10) **Patent No.:** **US 9,272,022 B2**
(45) **Date of Patent:** ***Mar. 1, 2016**

(54) **COMPOSITIONS AND METHODS FOR
TREATING CANCER AND MODULATING
STRESS GRANULE FORMATION**

(75) Inventors: **Paul Chang**, Cambridge, MA (US);
Sejal K. Vyas, Somerville, MA (US);
Anthony Leung, Somerville, MA (US);
Phillip A. Sharp, Newton, MA (US)

(73) Assignee: **Massachusetts Institute of Technology**,
Cambridge, MA (US)

(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 52 days.

This patent is subject to a terminal dis-
claimer.

(21) Appl. No.: **13/435,932**

(22) Filed: **Mar. 30, 2012**

(65) **Prior Publication Data**

US 2013/0156776 A1 Jun. 20, 2013

Related U.S. Application Data

(63) Continuation of application No. 12/822,995, filed on
Jun. 24, 2010, now abandoned.

(60) Provisional application No. 61/269,614, filed on Jun.
26, 2009.

(51) **Int. Cl.**

A61K 38/47 (2006.01)
A61K 39/395 (2006.01)
C12N 15/113 (2010.01)
C07K 16/40 (2006.01)
A61K 31/713 (2006.01)
A61K 38/45 (2006.01)
C07H 21/02 (2006.01)
C12Q 1/68 (2006.01)
G01N 33/68 (2006.01)

(52) **U.S. Cl.**

CPC **A61K 38/47** (2013.01); **A61K 31/713**
(2013.01); **A61K 38/45** (2013.01); **A61K**
39/395 (2013.01); **A61K 39/3955** (2013.01);
C07H 21/02 (2013.01); **C07K 16/40** (2013.01);
C12N 15/113 (2013.01); **C12N 15/1137**
(2013.01); **C12Q 1/6886** (2013.01); **G01N**
33/6893 (2013.01); **C12N 2310/14** (2013.01);
C12N 2320/12 (2013.01); **G01N 2333/91142**
(2013.01); **G01N 2500/02** (2013.01)

(58) **Field of Classification Search**

None
See application file for complete search history.

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Primary Examiner — Daniel E Kolker

Assistant Examiner — James Rogers

(74) *Attorney, Agent, or Firm* — Clark & Elbing LLP;
Kristina Bieker-Brady

(57) **ABSTRACT**

The invention provides methods for treating or decreasing the
likelihood of developing a stress-granule related disorder
and/or cancer by administering one or more poly-ADP-ribose
polymerase (PARP) inhibitors, one or more PARP activators,
one or more poly-ADP-ribose glycosylase (PARG) activa-
tors, and/or one or more poly-ADP-ribose glycohydrolase
ARH3 activators. The invention also provides corresponding
methods of decreasing stress granule formation and/or pro-
liferation in a cell or a population of cells. The invention
further provides methods of increasing the number of stress
granules and proliferation in a cell or a population of cells by
administering one or more PARP activators, one or more
PARP inhibitors, one or more PARG inhibitors, and/or one or
more ARH3 inhibitors. The invention also provides methods
for screening for agents for treating or decreasing the likeli-
hood of developing a stress granule-related disorder or can-
cer, and methods for determining the propensity for develop-
ing a stress granule-related disorder or cancer, as well as
compositions and kits containing one or more PARP inhibi-
tors, one or more PARP activators, one or more PARG acti-
vators, and one or more ARH3 activators.

8 Claims, 31 Drawing Sheets
(11 of 31 Drawing Sheet(s) Filed in Color)

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Figure 1

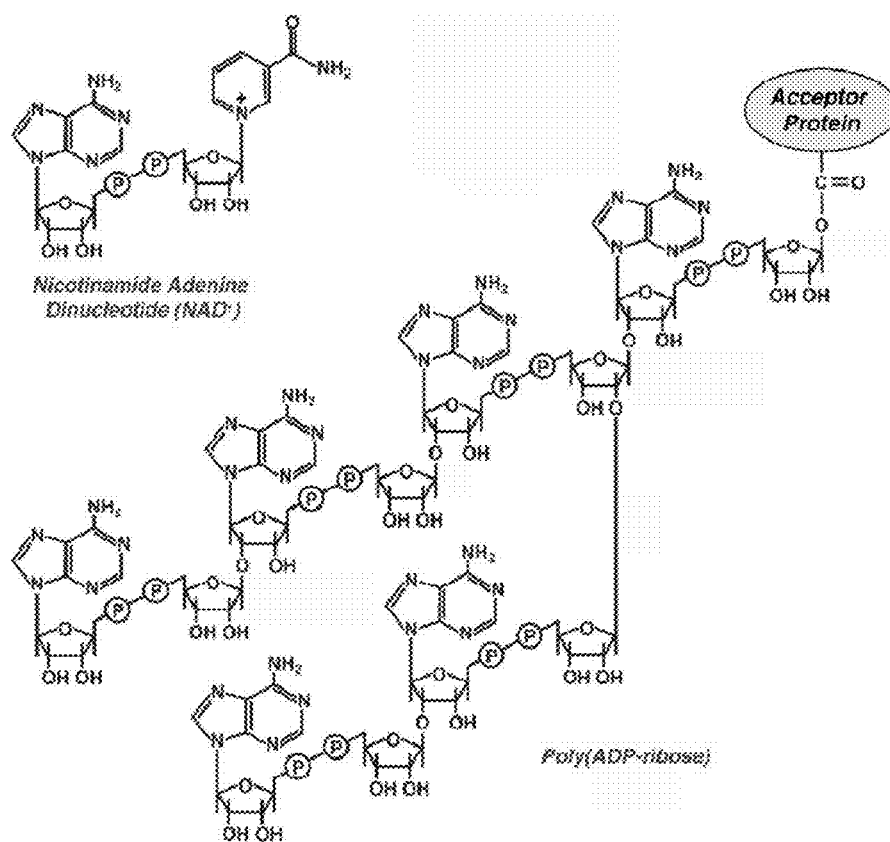


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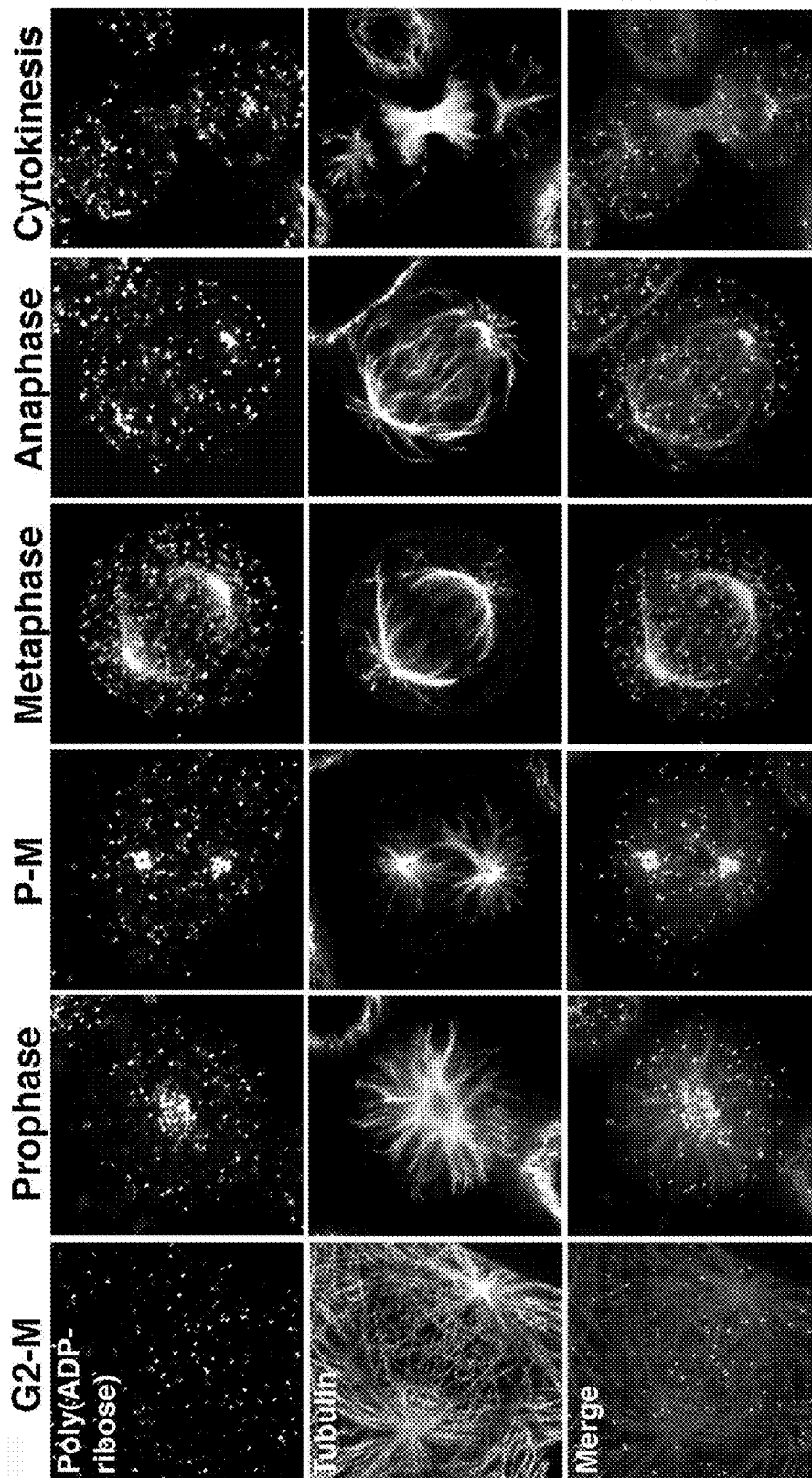


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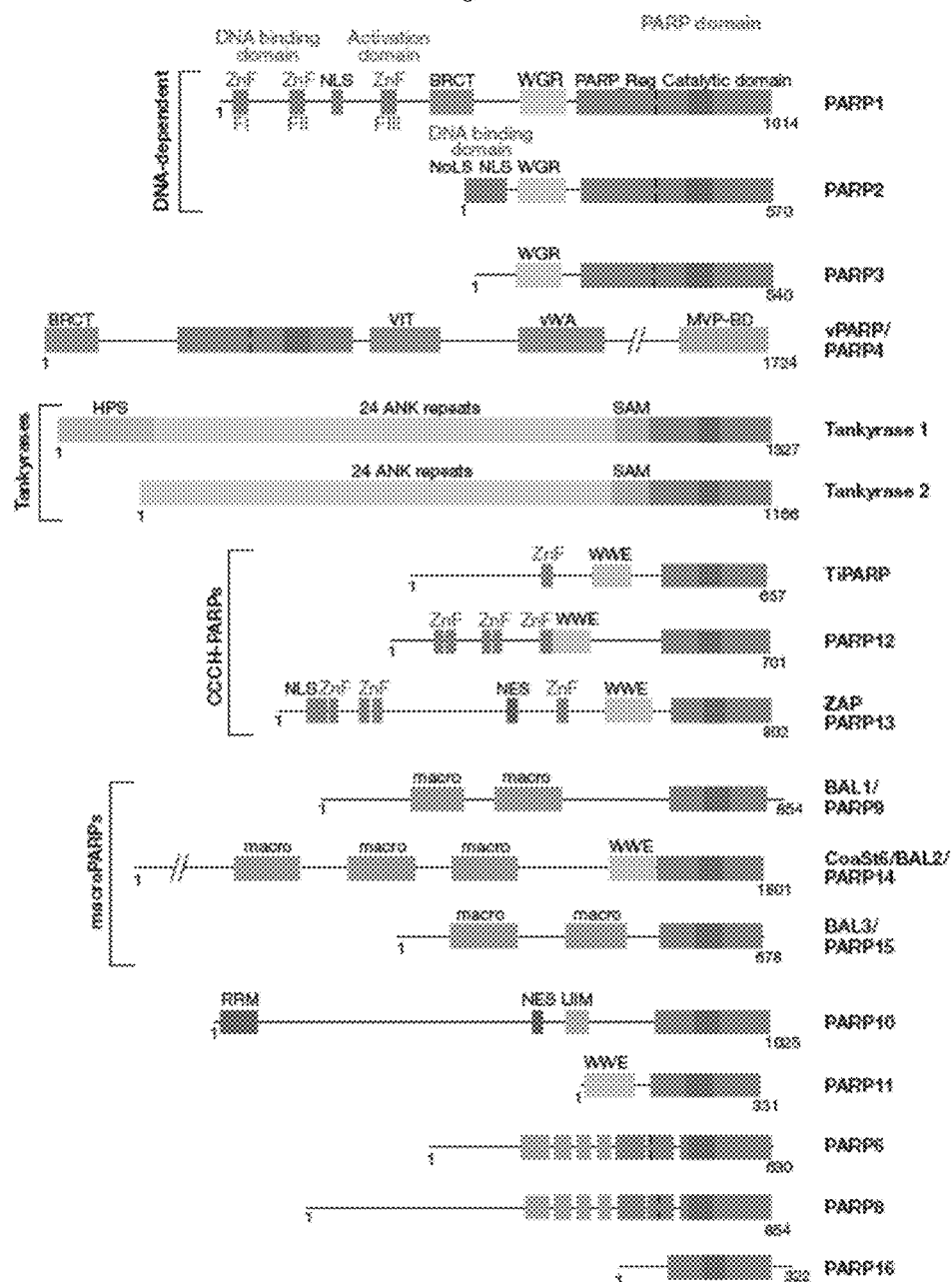
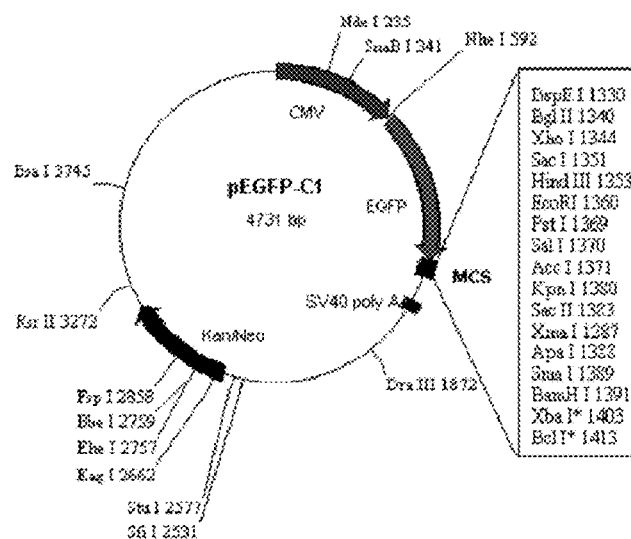


Figure 4

pEGFP-C1

Polylinker Sequence (SEQ ID NO: 29):

```

CTGTACARGTCCGGACTCAGATCTCCAGCTCAAGCTTGAATTCTGCAGTCGAC 1374
      EspEI      Bgl II XbaI SacI HindIII EcoRI PstI Sall/AccI

CCFACCCCGGGGGGCGCCCATCCACCCCATCTACATAACTGATCATTAATCAGCCAT 1428
      KpnI Sac II Apa I      BamHI      Xba I      Bcl I
              Xma I/Sma I

```

Figure 5

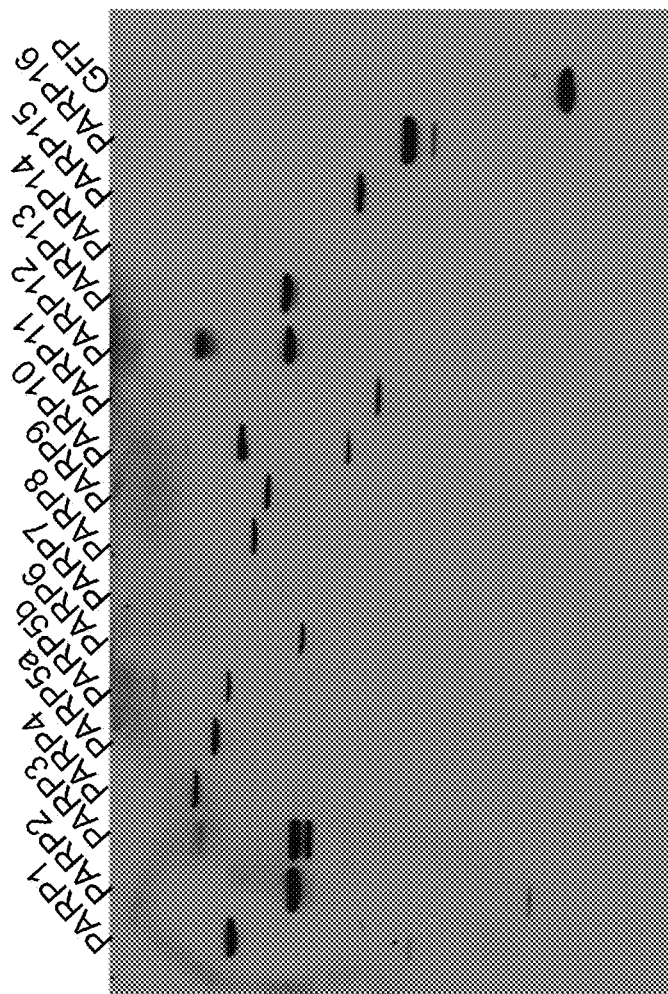
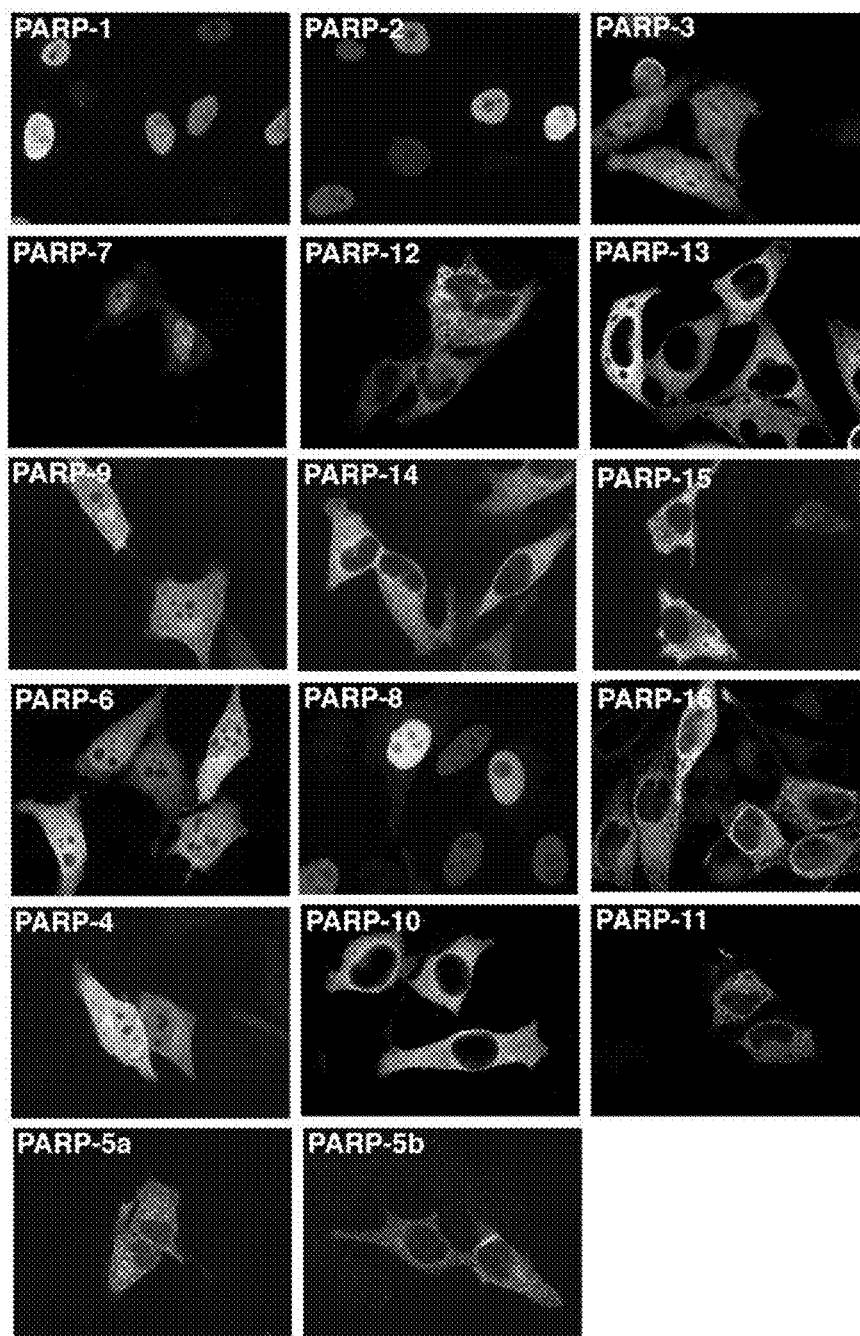


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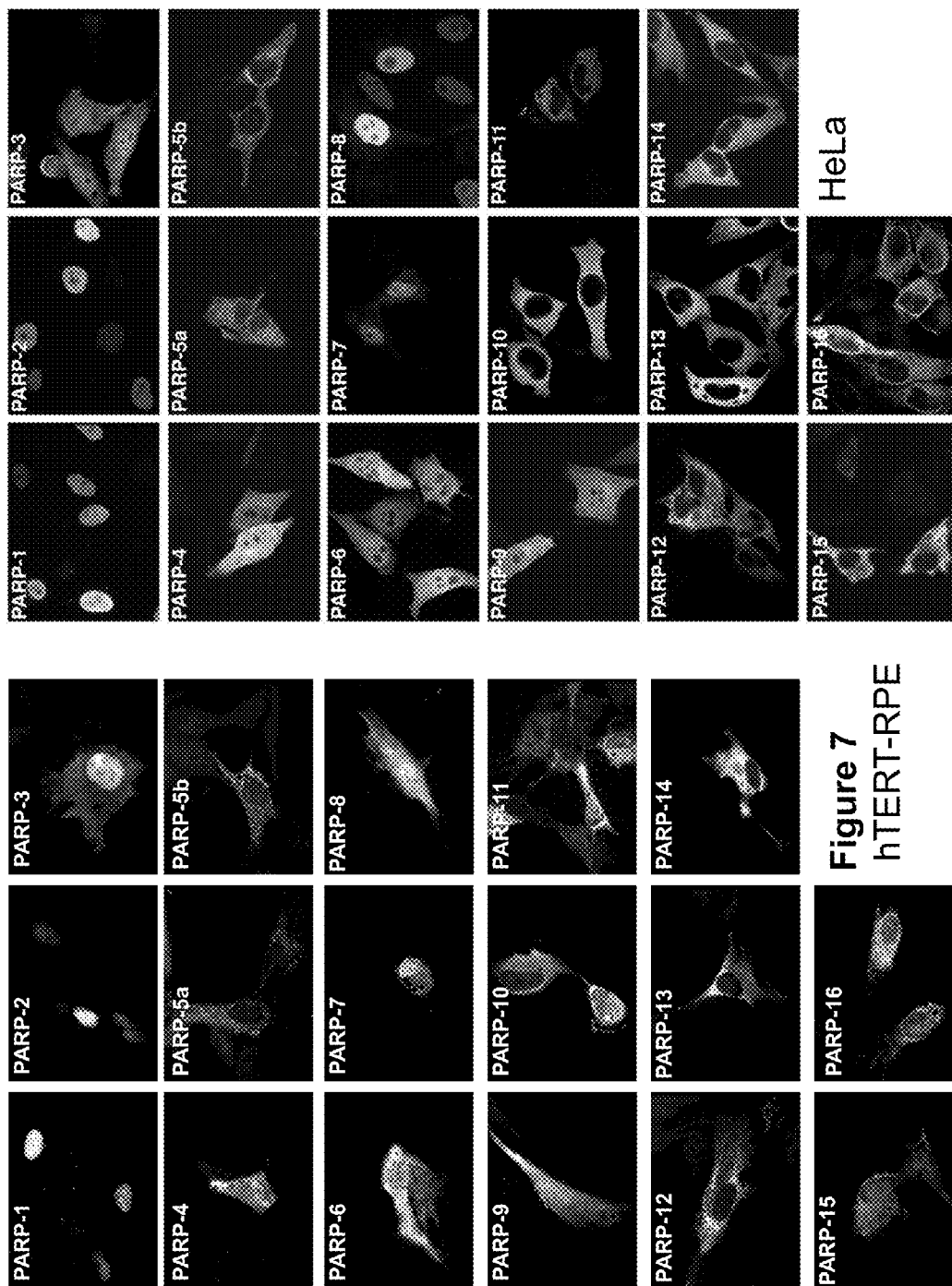


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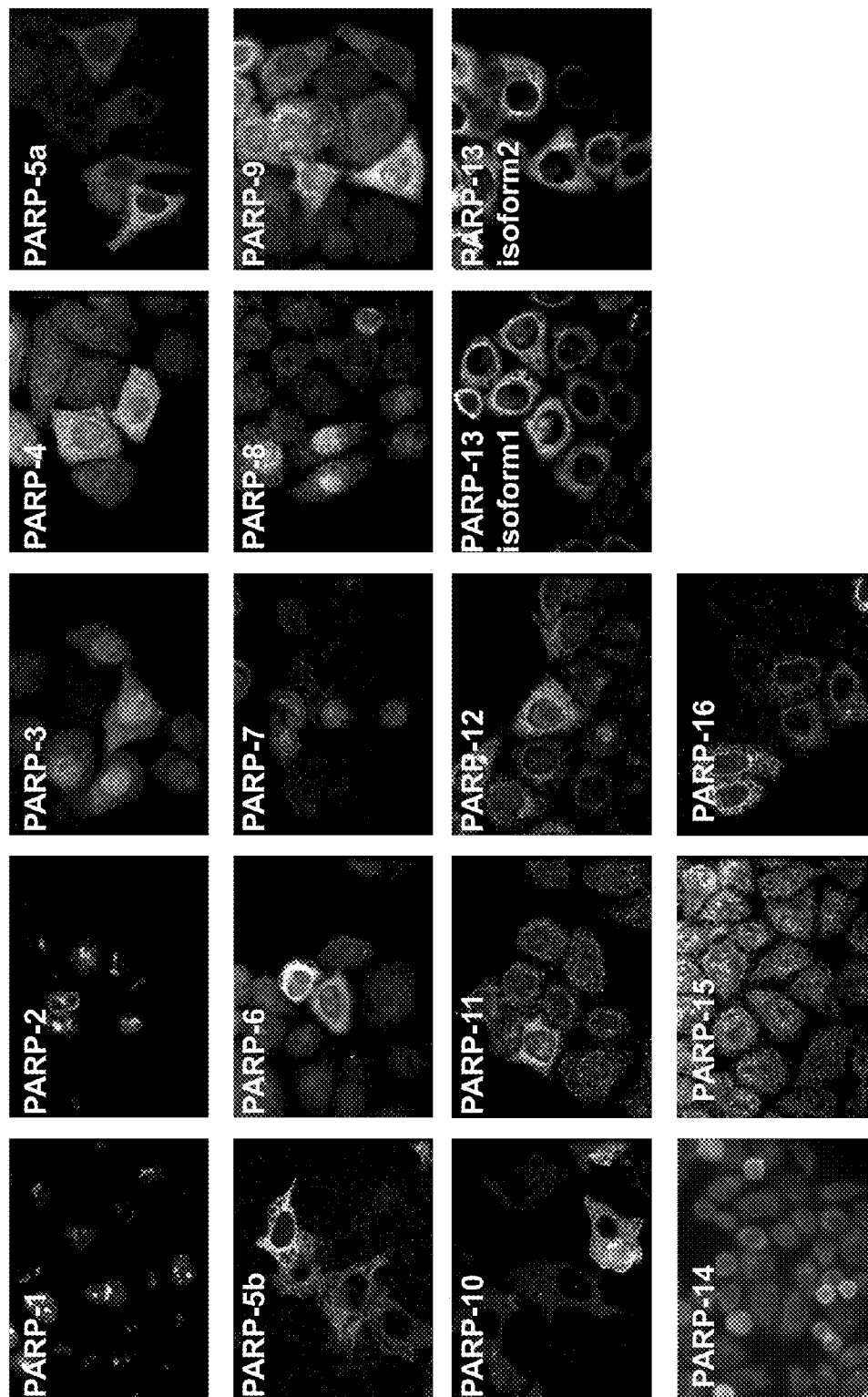


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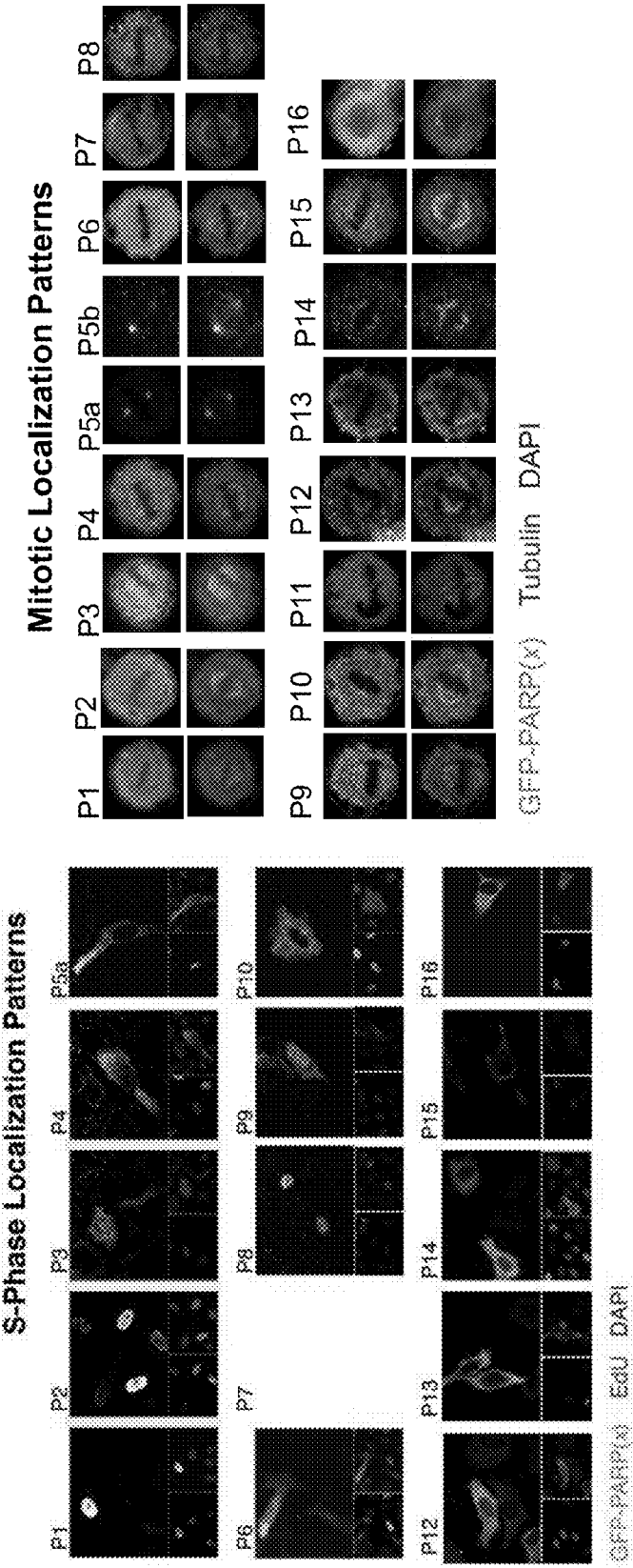


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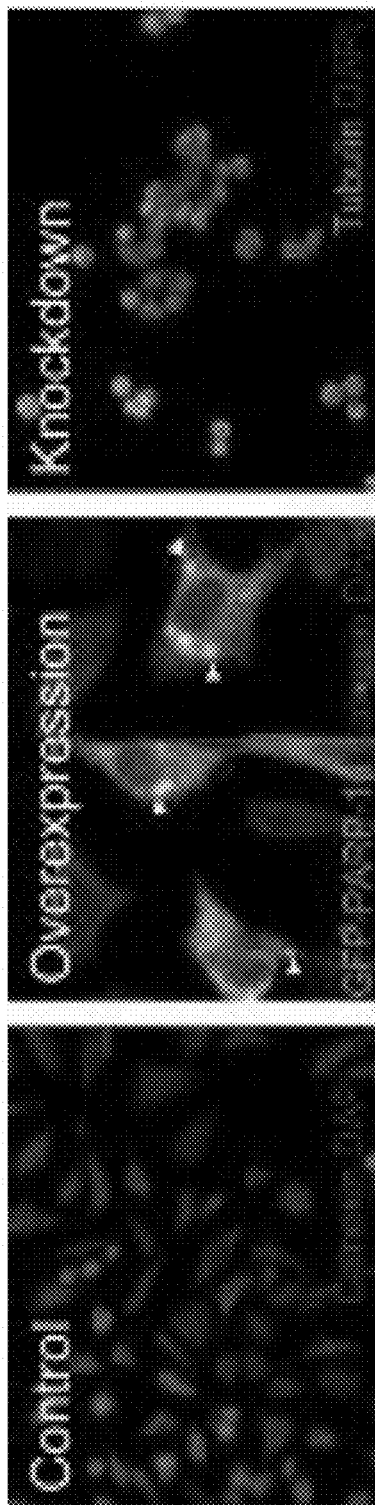
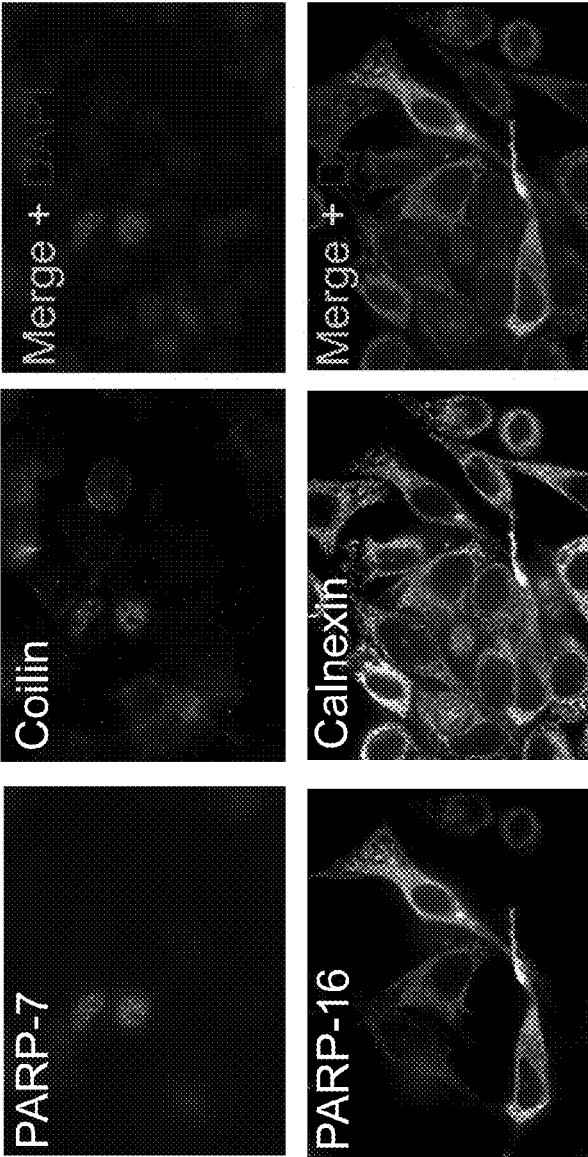


Figure 11

Marker	Organelle
Actin	Cytoskeleton
ATP Synthase	Mitochondria
ADP Ribosylation Factor	TGN/ Secretory Vesicles
Calnexin	ER
EEA1	Early Endosome
Gamma Tubulin	Centrosome
GW182	GW Bodies
Lamin B1	Nuclear Envelope
LAMP2	Lysosome
Mannosidase II	Golgi
Tubulin	Cytoskeleton
Catalase	Peroxisomes
PSP-1	Para Speckles
H2AX	Histones
Coilin	Cajal Bodies



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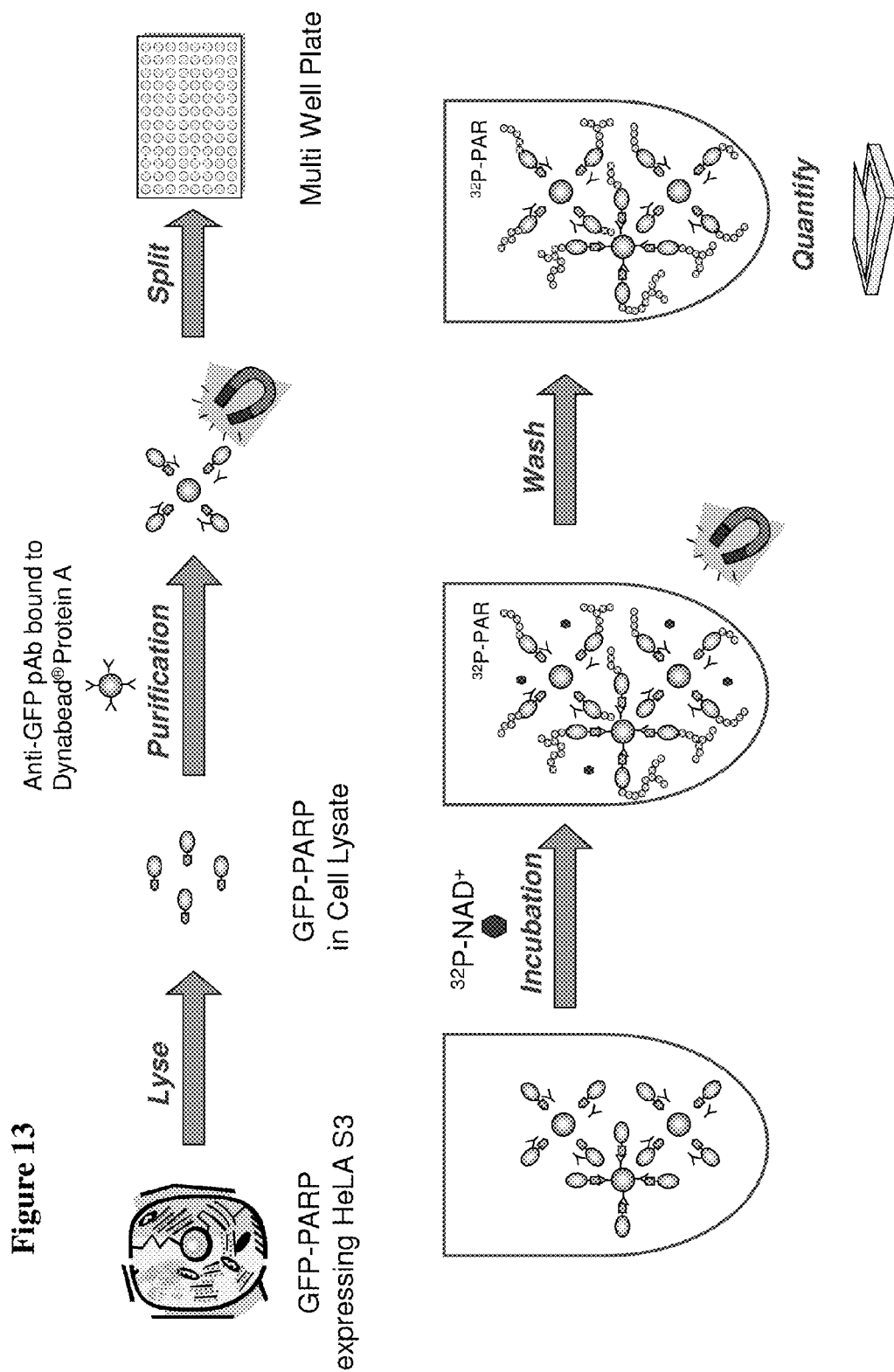


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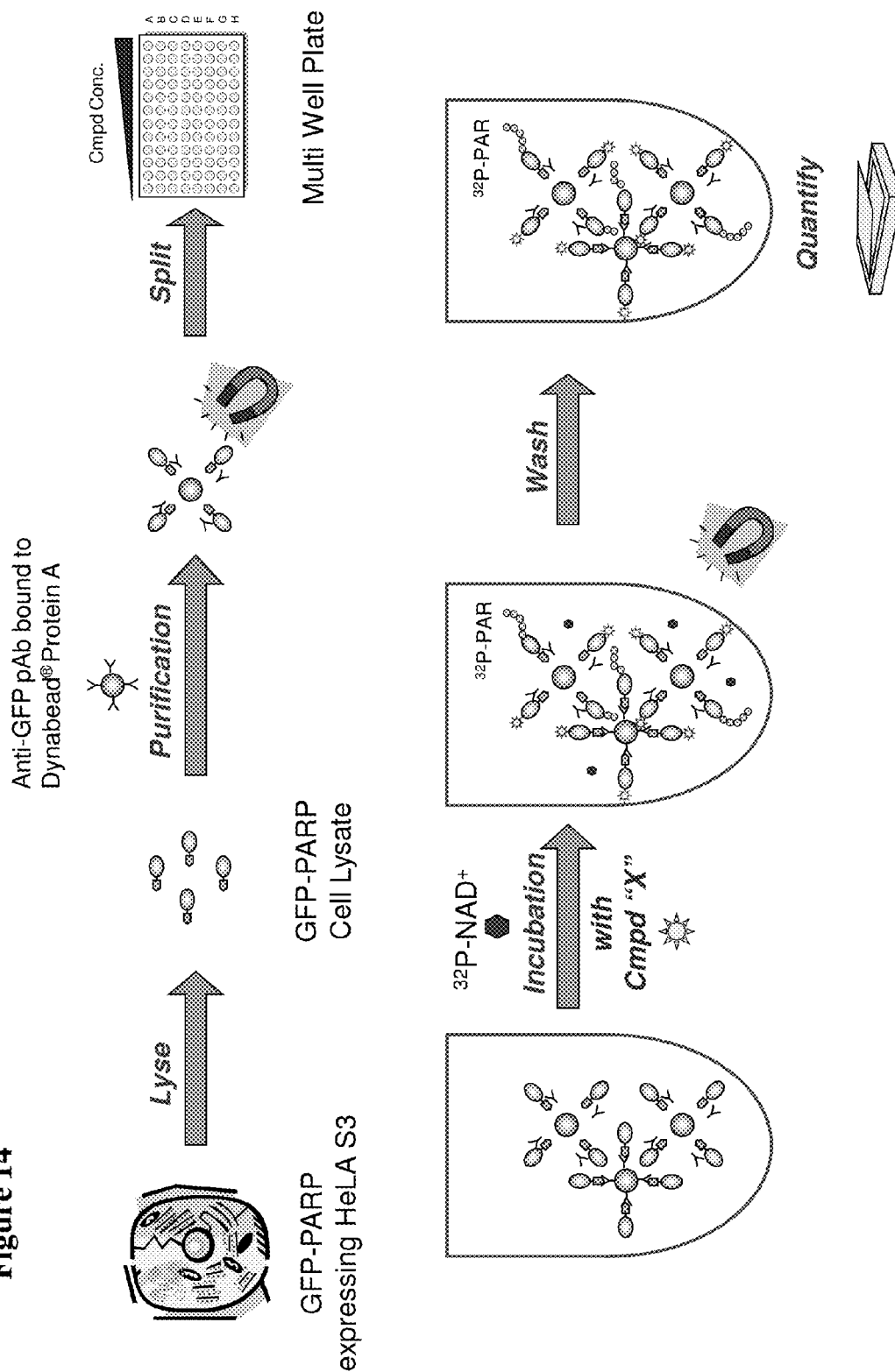


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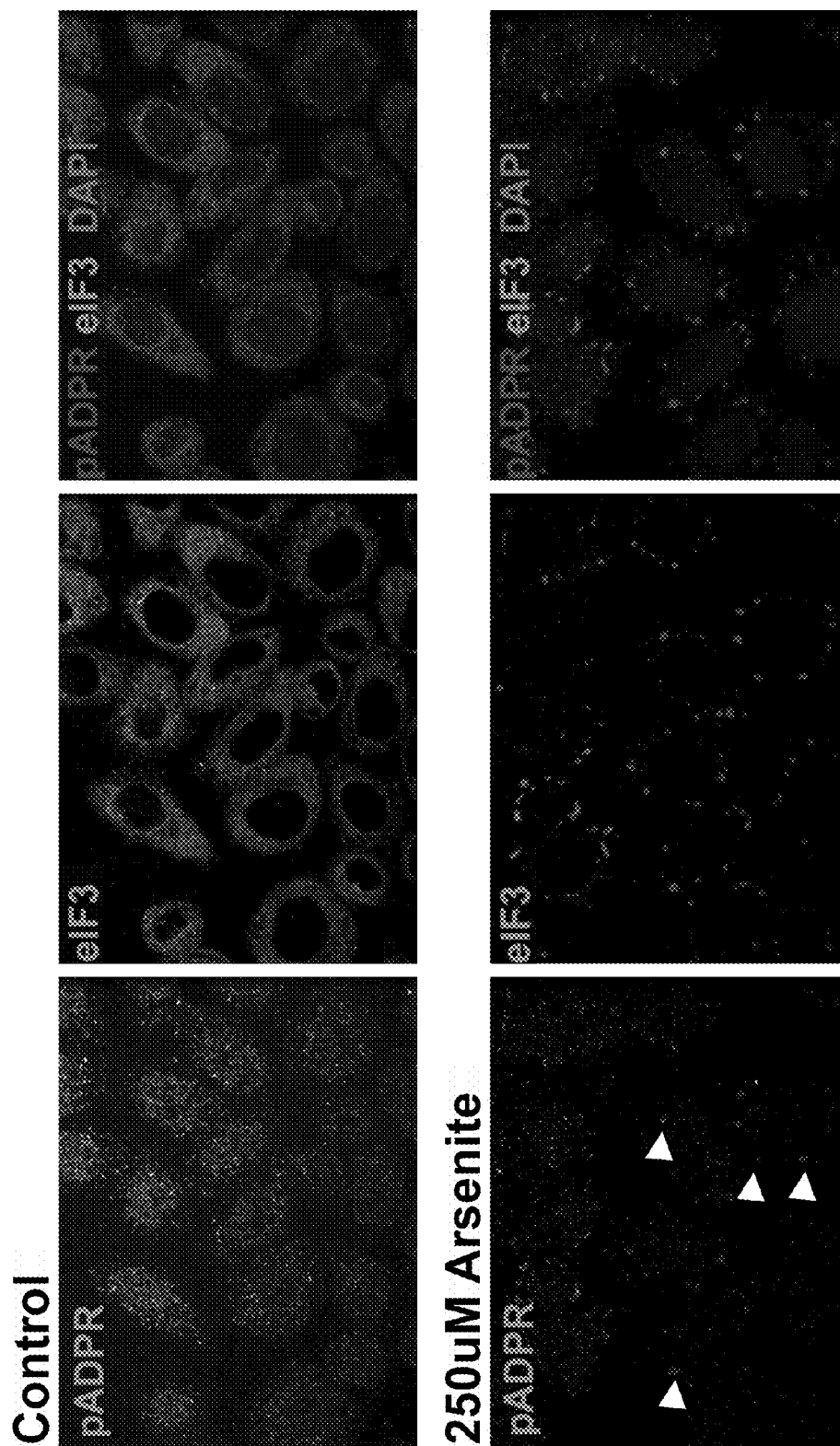


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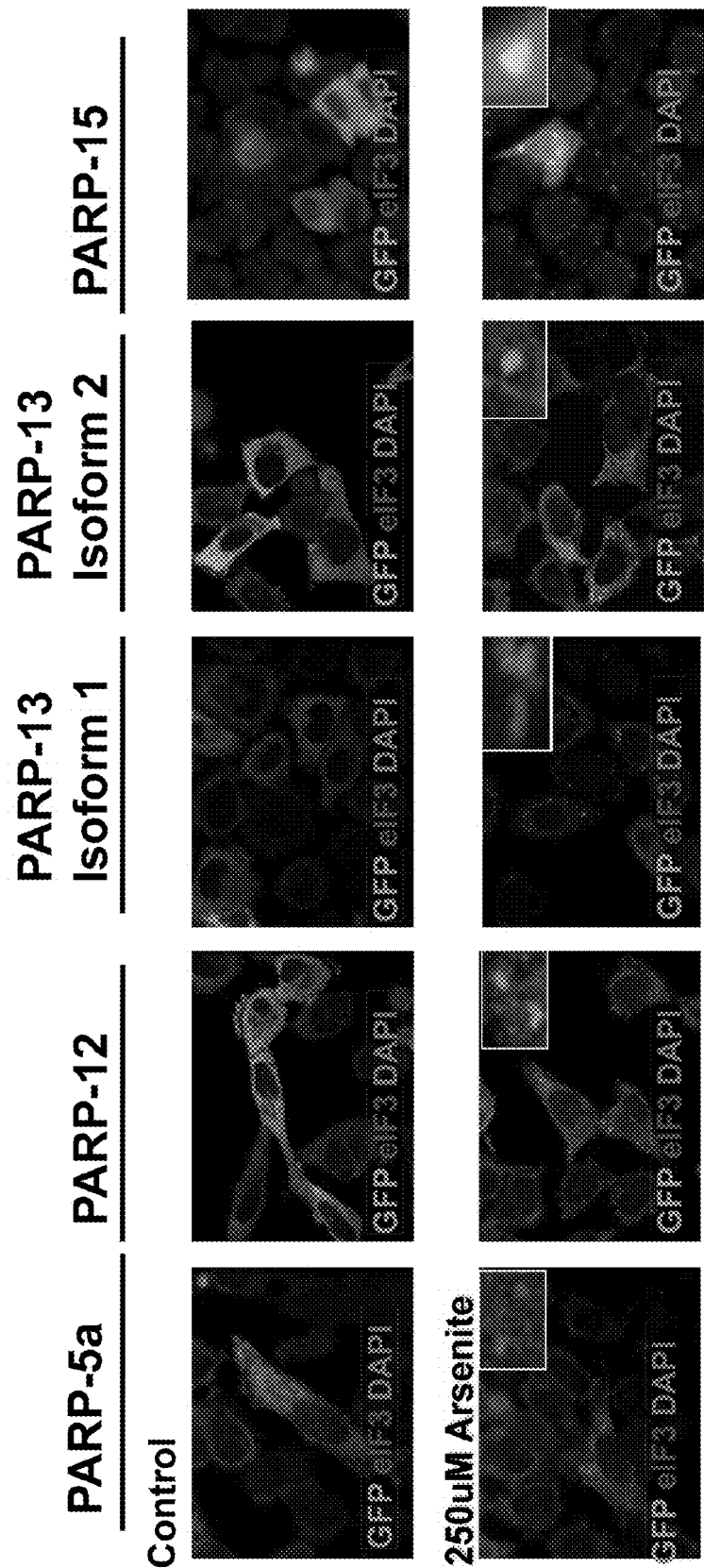


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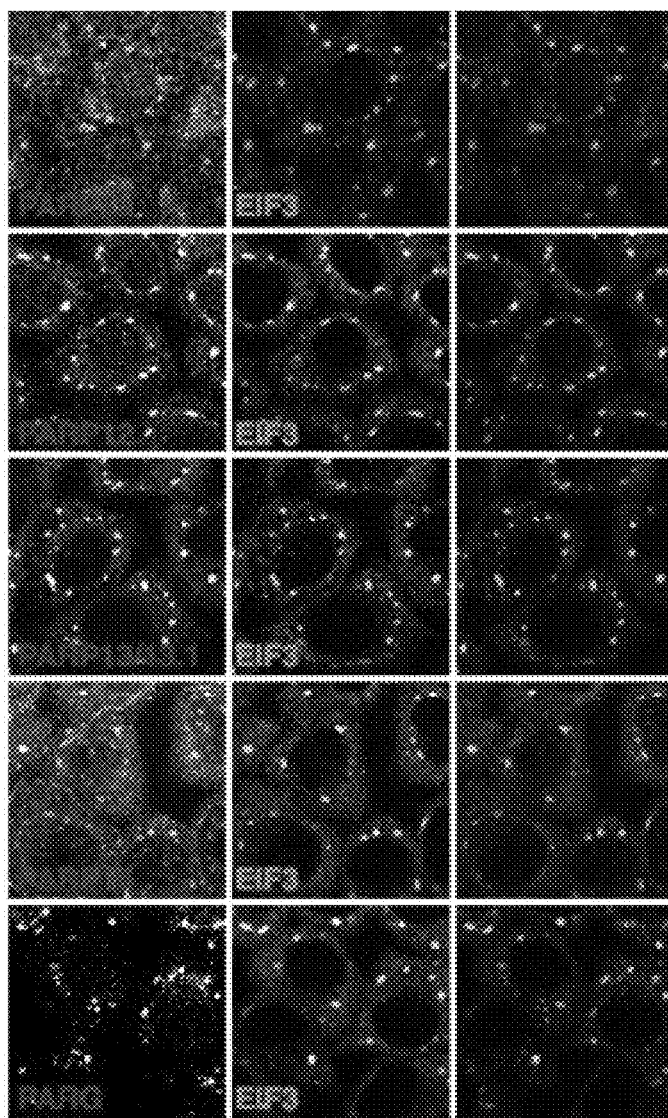
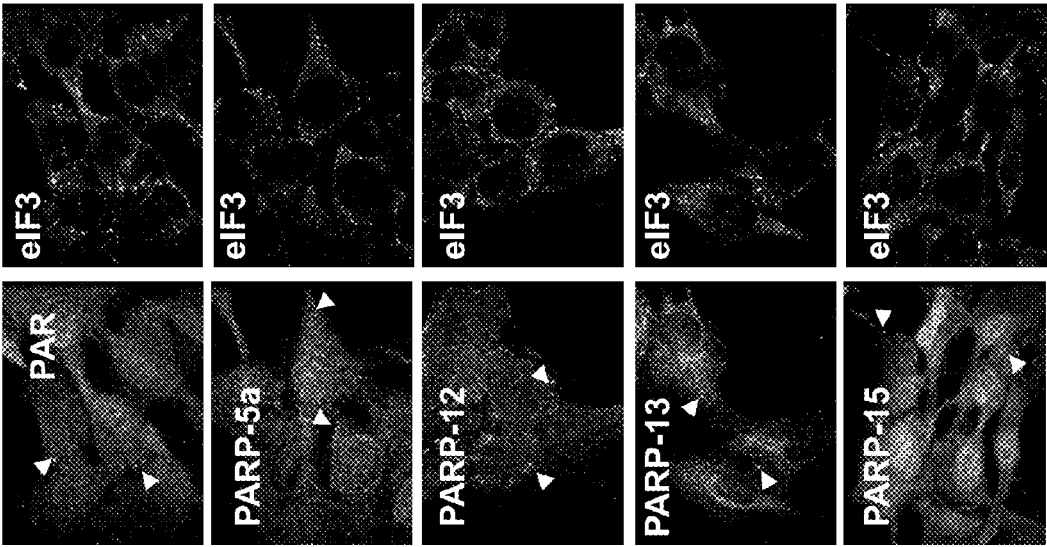


Figure 19
hTERT-RPE



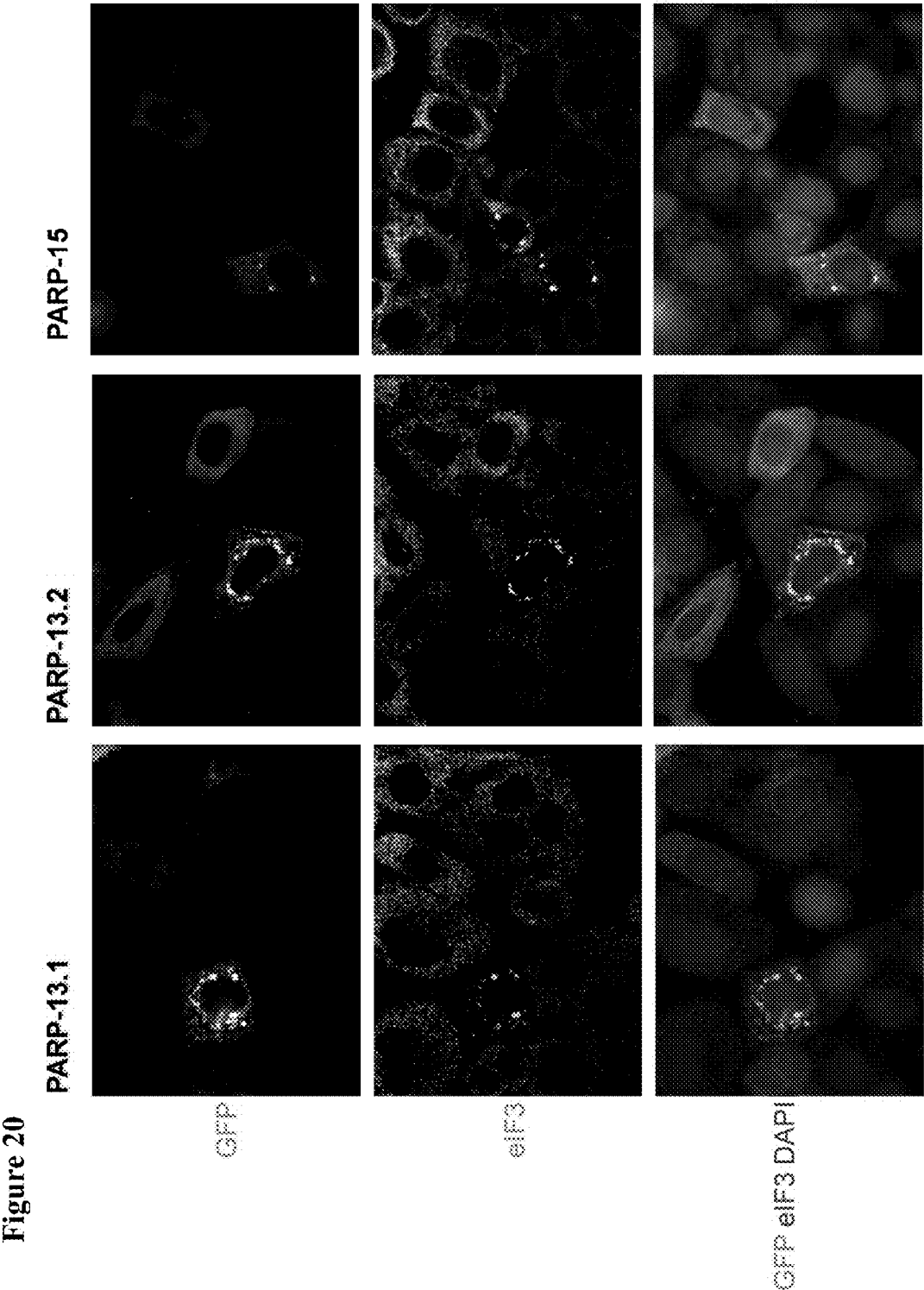


Figure 21

eIF3 PARP-11-GFP DAPI

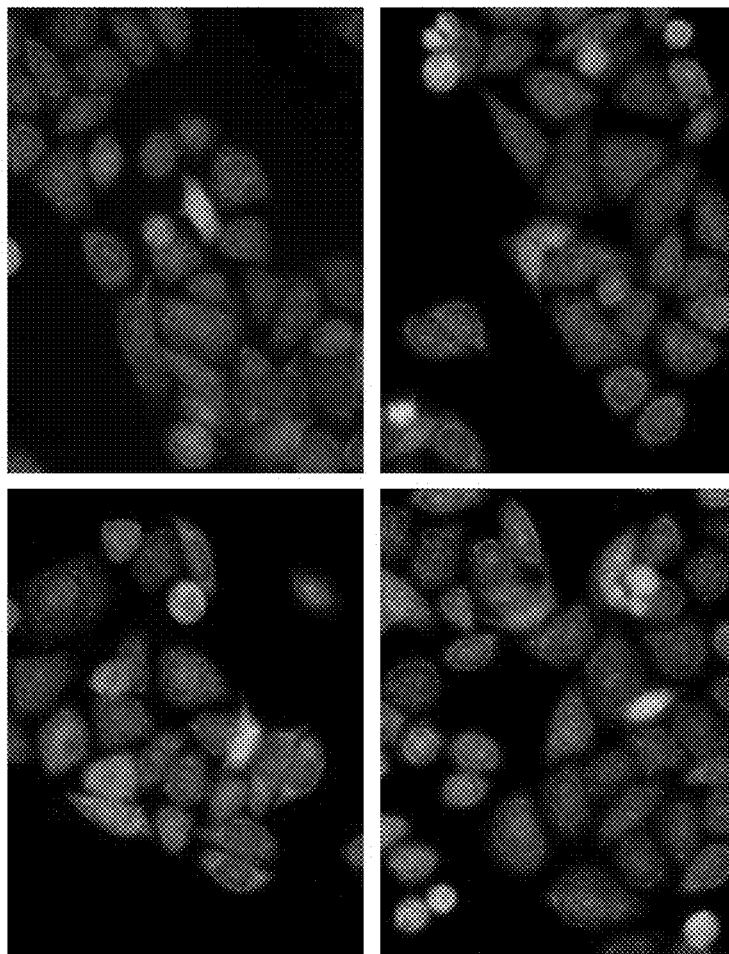


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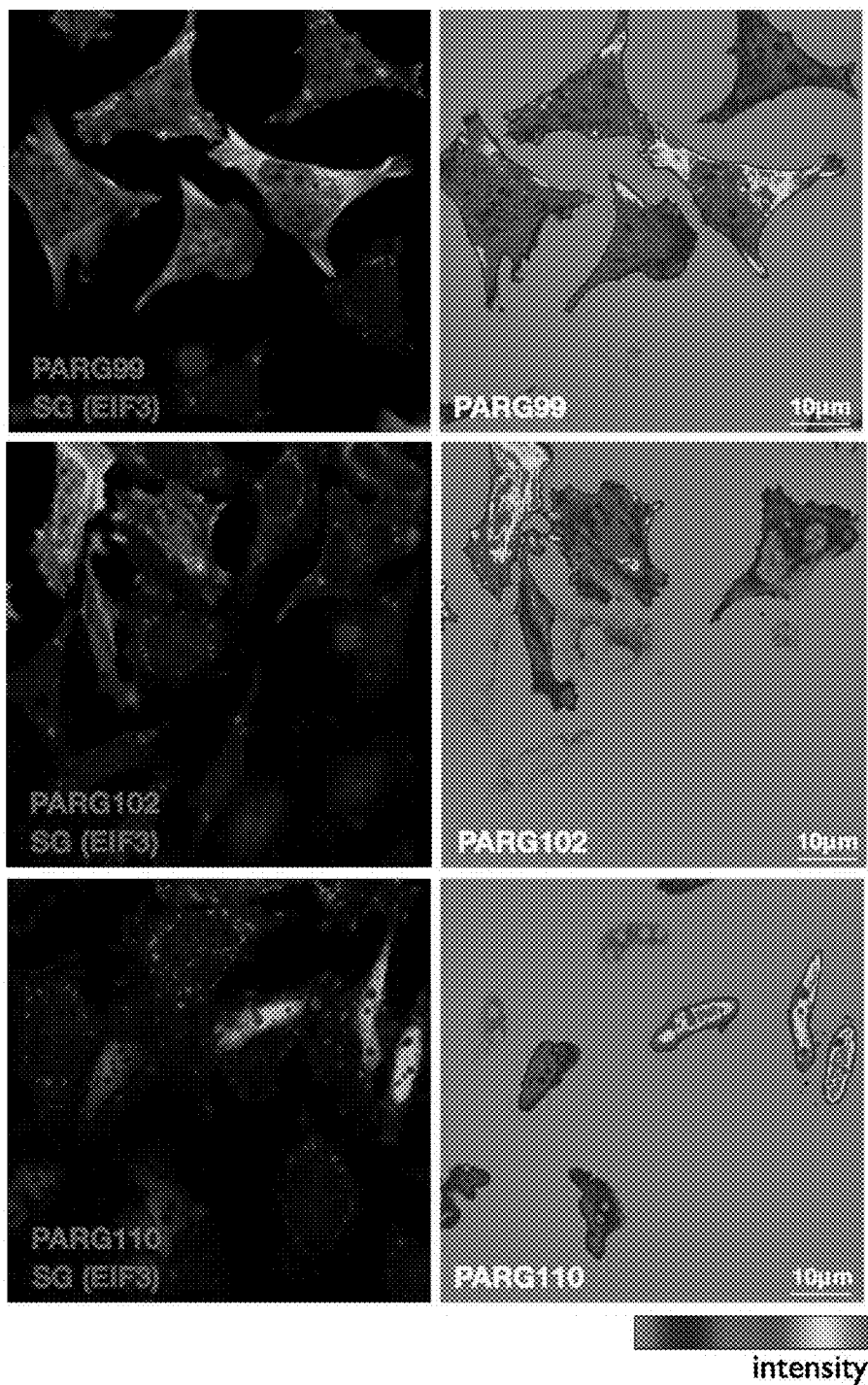
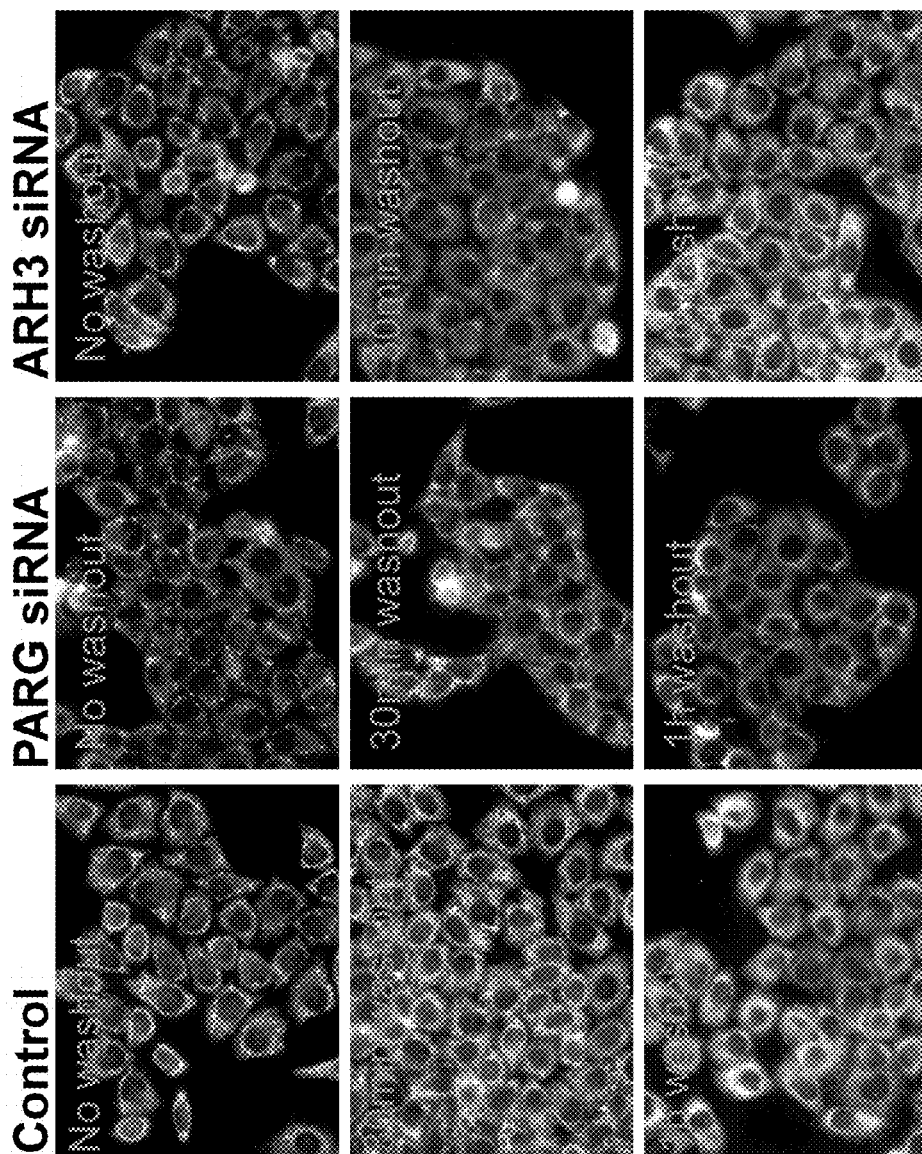


Figure 23



PARG siRNA
ARH3 siRNA
control siRNA

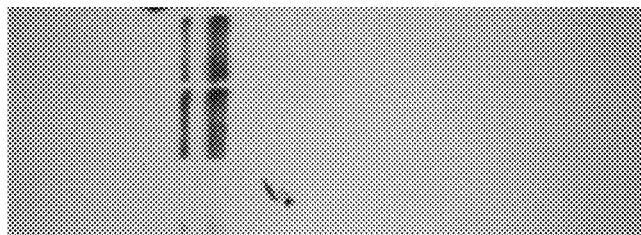


Figure 24

Stress Granule Disassembly Kinetics

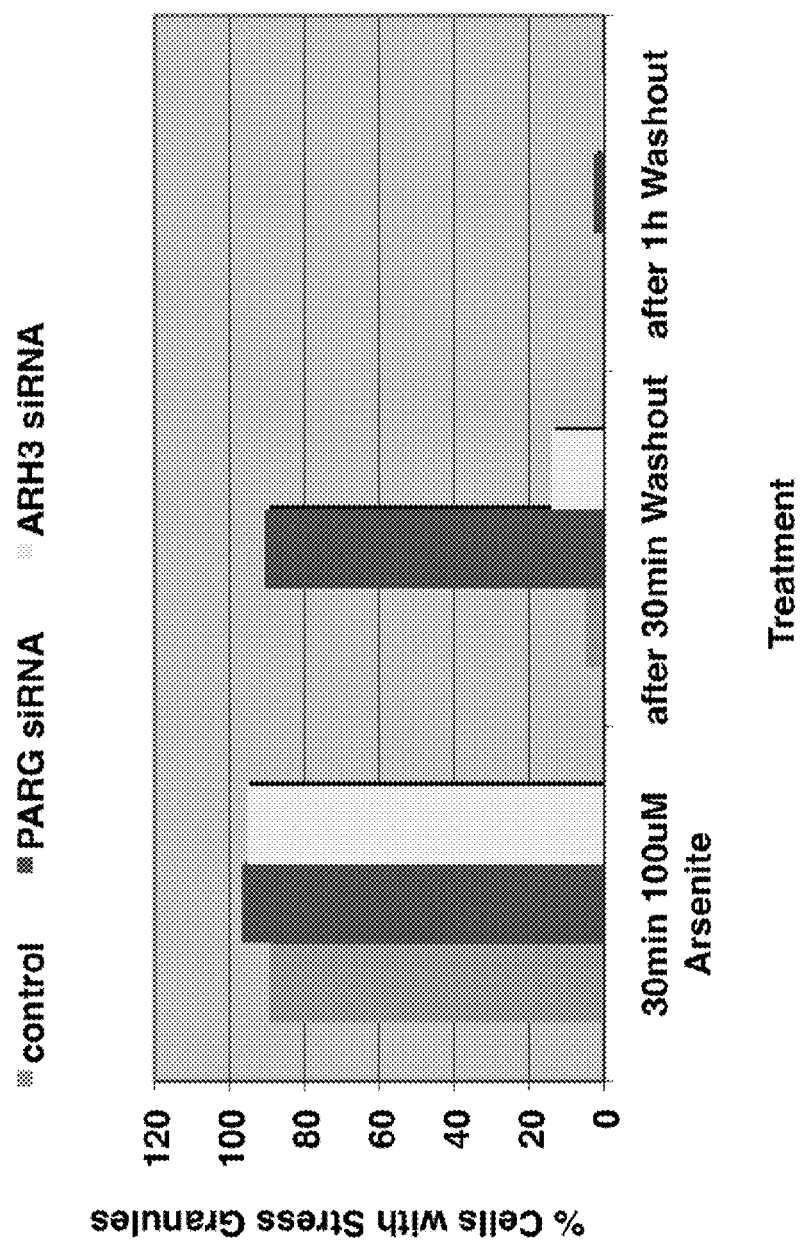


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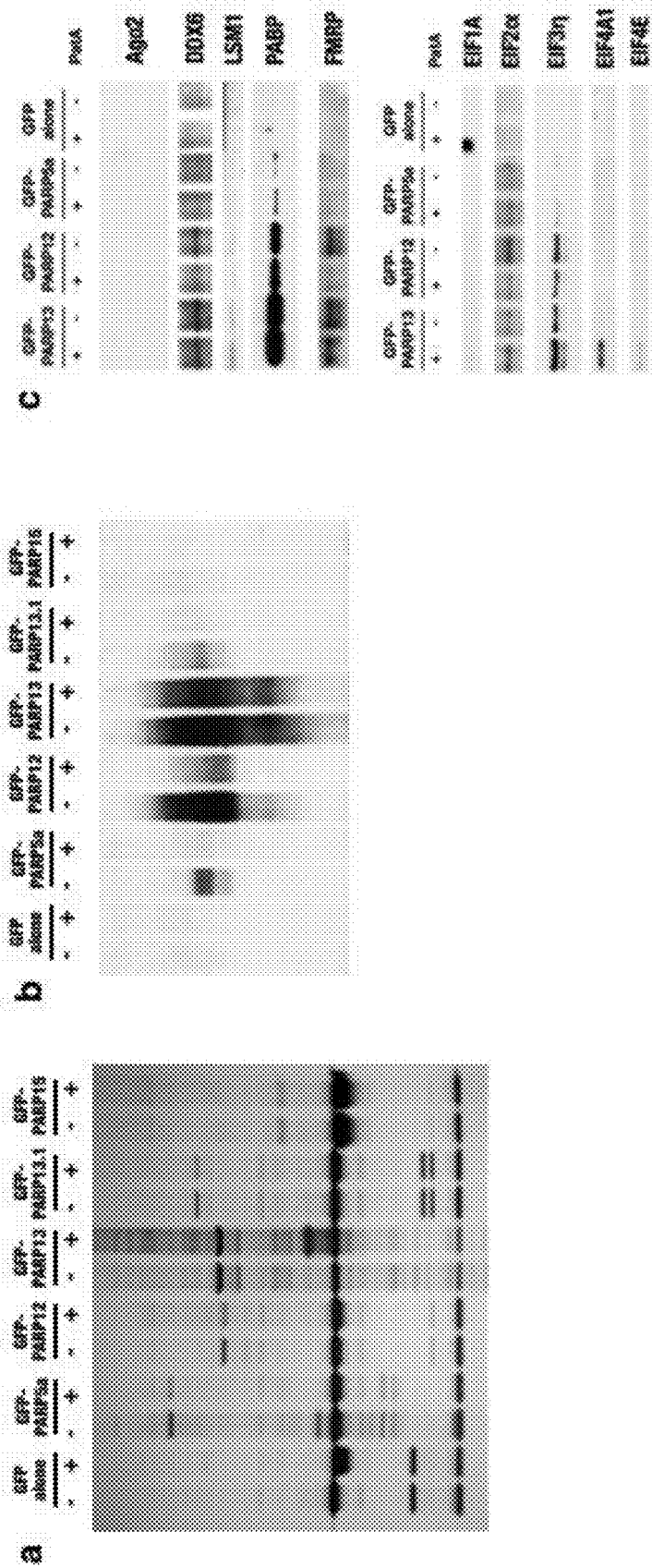


Figure 26

G3BP Structure

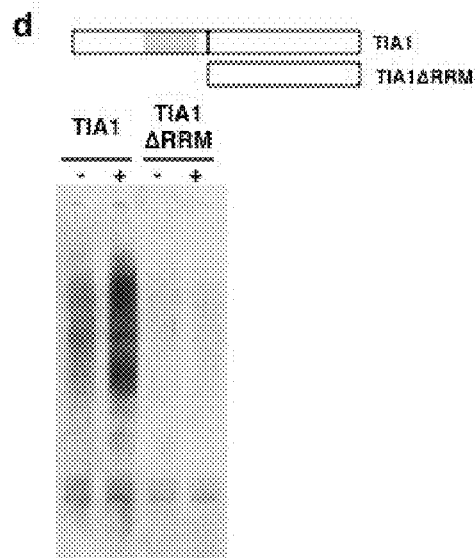
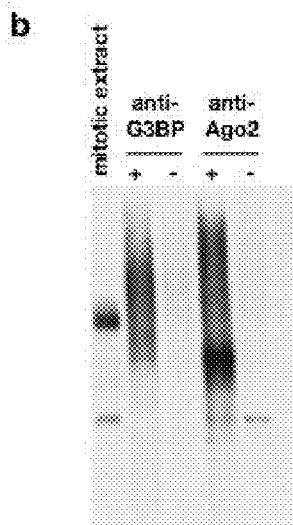
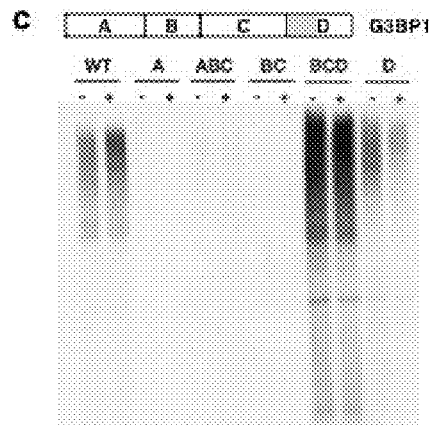
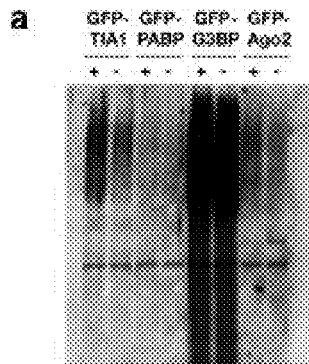
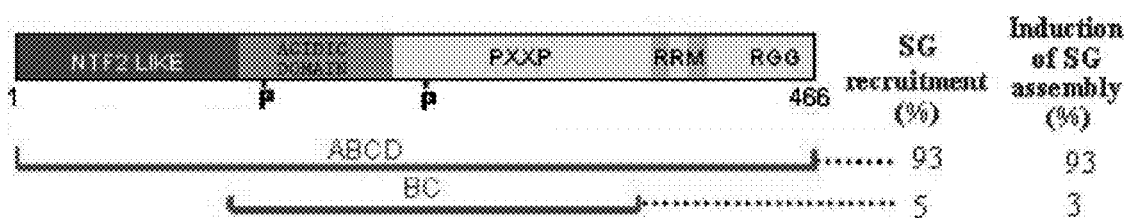


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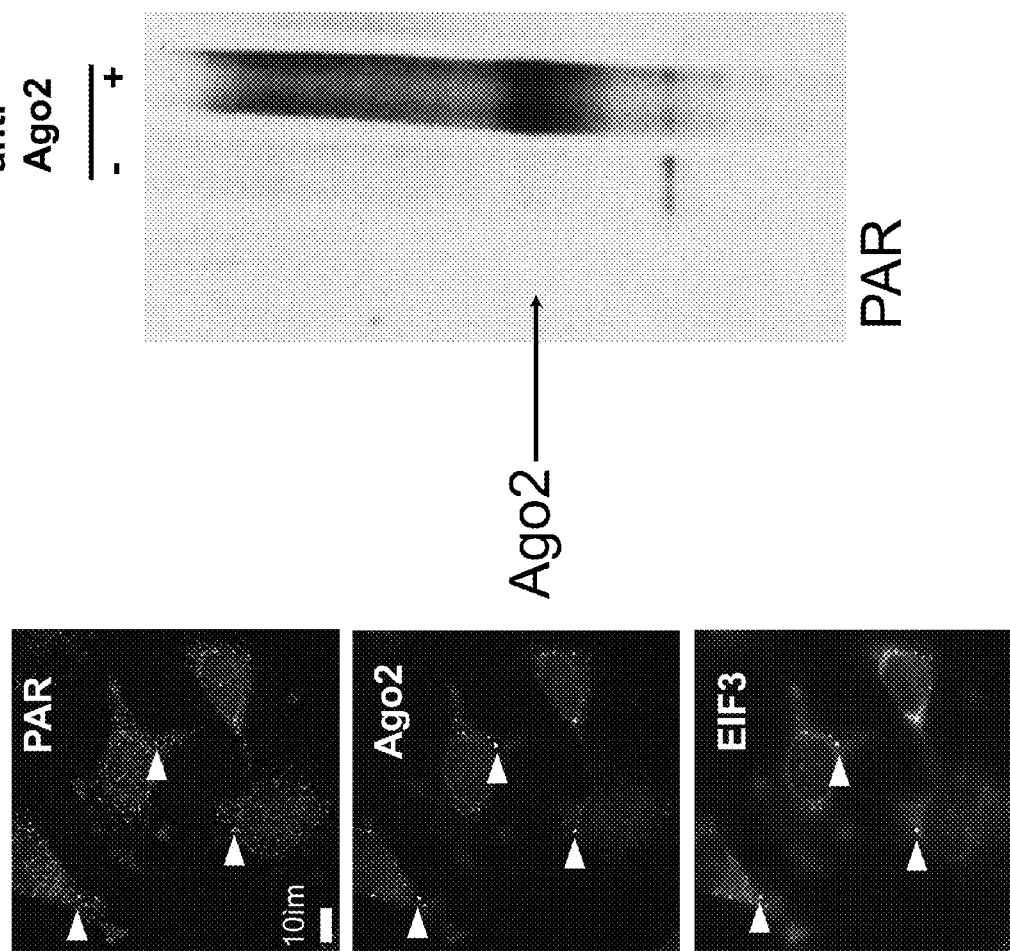


Figure 28

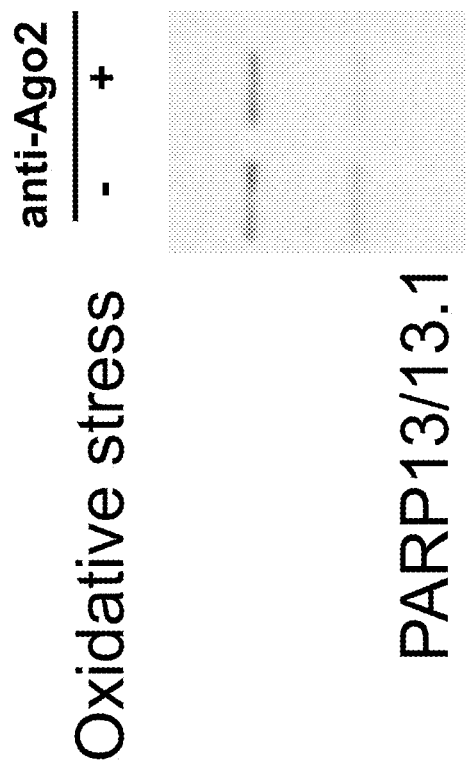
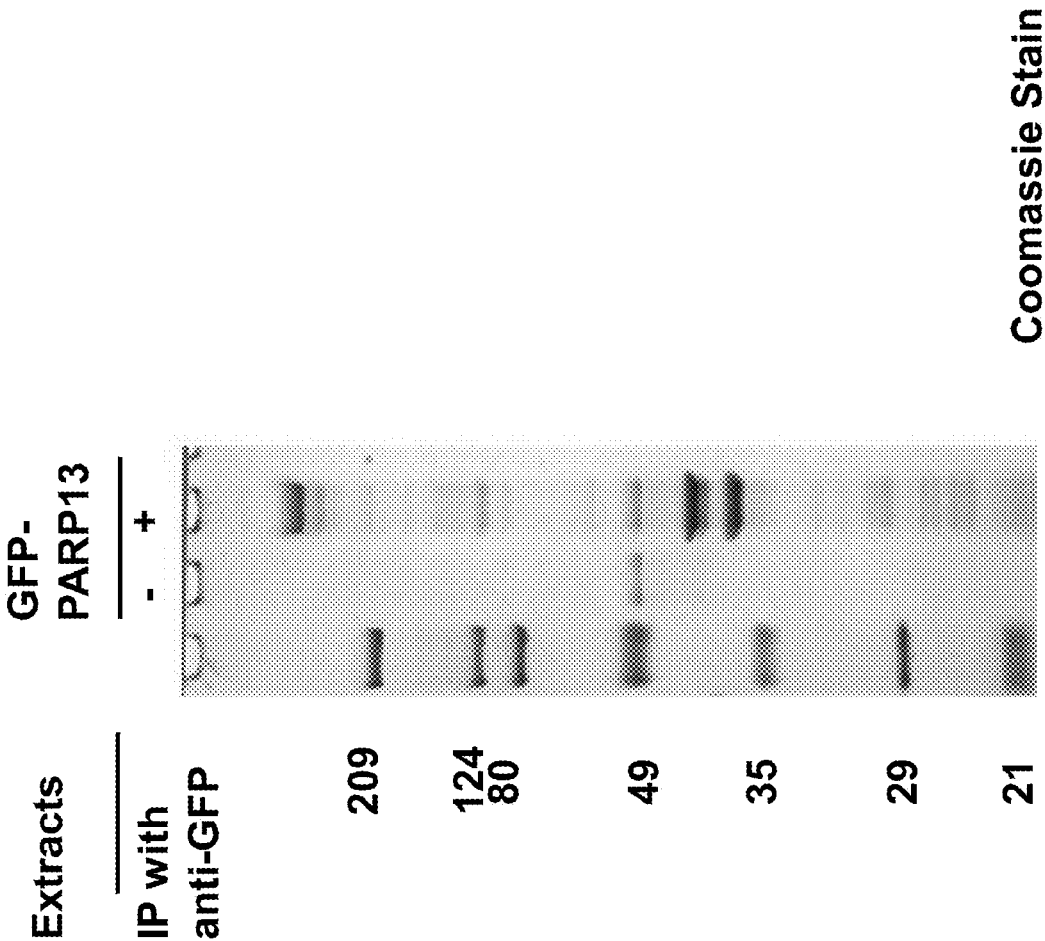


Figure 29



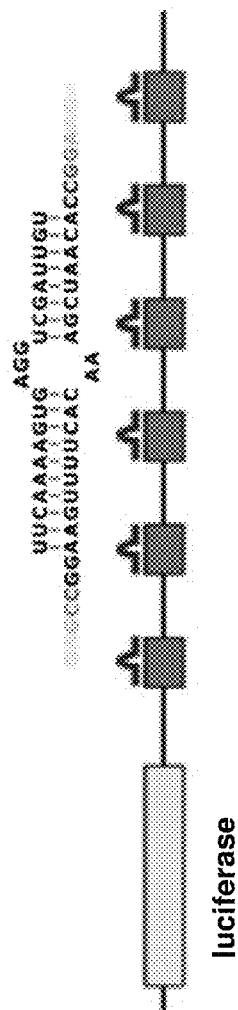
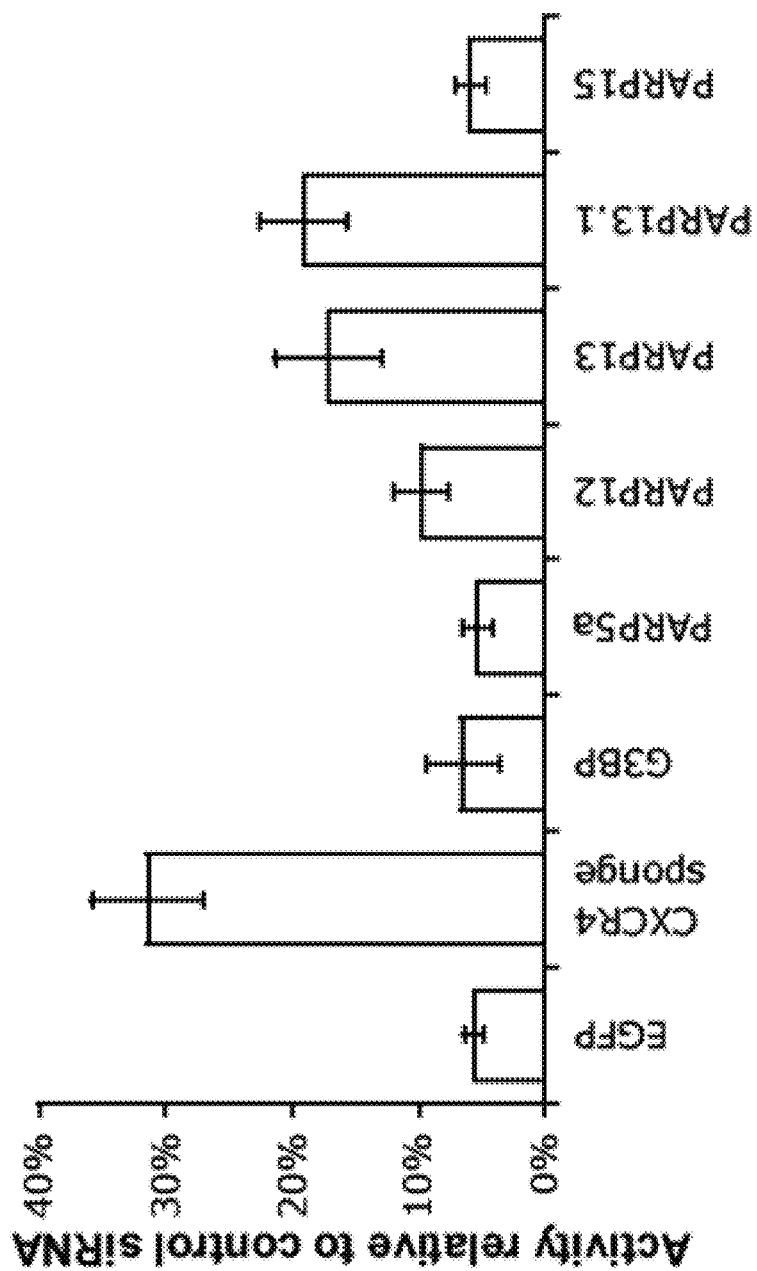
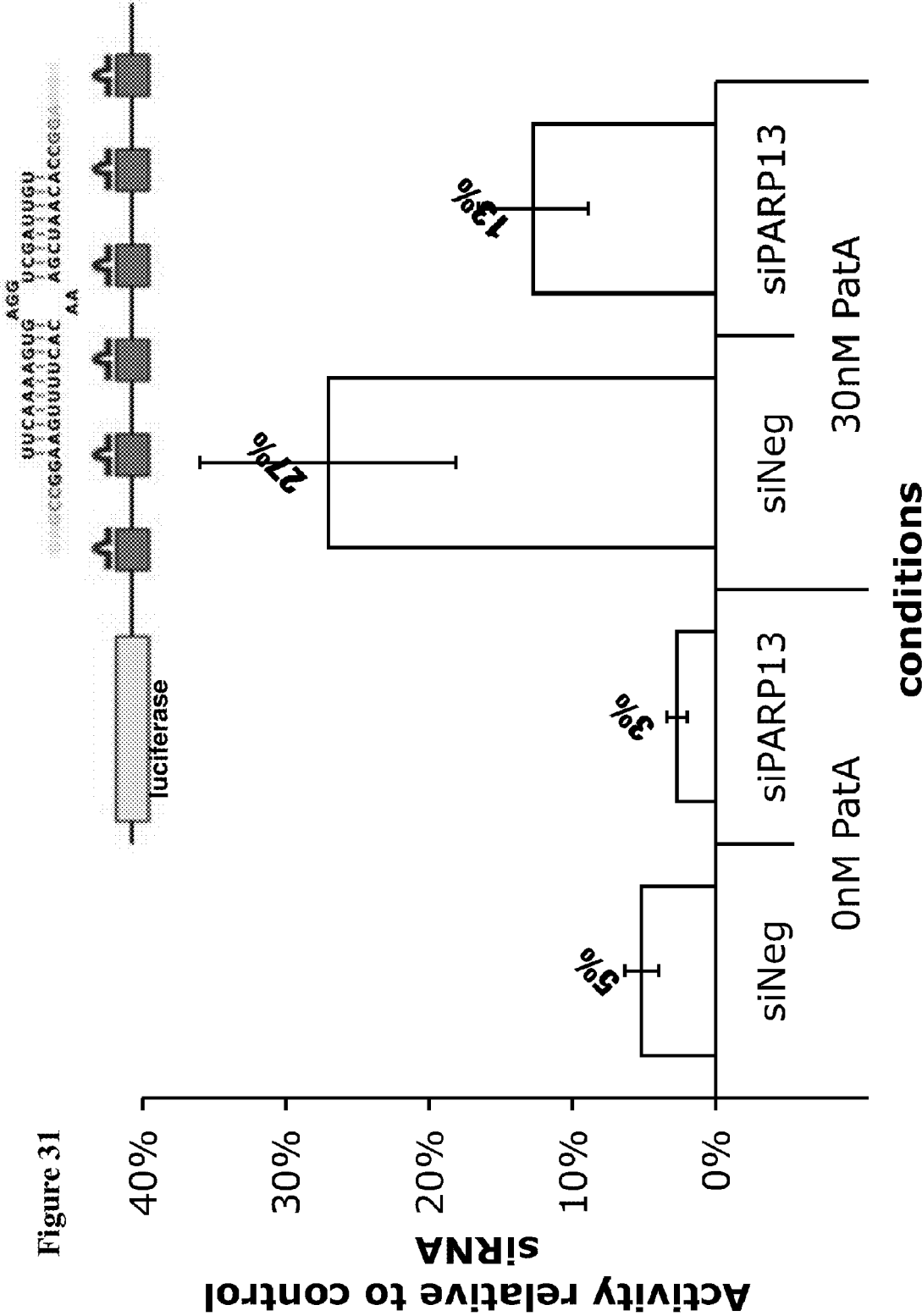


Figure 30





1

COMPOSITIONS AND METHODS FOR TREATING CANCER AND MODULATING STRESS GRANULE FORMATION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 12/822,995, filed Jun. 24, 2010, which claims the benefit of the filing date of U.S. Provisional Application No. 61/269,614, filed Jun. 26, 2009, each of which is herein incorporated by reference.

STATEMENT AS TO FEDERALLY FUNDED RESEARCH

This invention was made with government support under grant numbers R01 CA133404 and P01 CA042063 awarded by the National Institutes of Health. The government has certain rights in this invention.

FIELD OF THE INVENTION

The present invention relates to the field of molecular biology and molecular medicine.

BACKGROUND OF THE INVENTION

Poly-adenosine diphosphate (ADP)-ribose (PAR) polymers are the product of post-translational modifications carried out by PAR polymerases (PARPs). PAR is polymerized by PARPs onto acceptor proteins using nicotinamide adenine dinucleotide (NAD⁺) as substrate (FIG. 1). PAR polymers are localized to distinct cellular structures in different phases of the cell cycle and localize to the mitotic spindle during mitosis (FIG. 2). There are at least 18 PARPs in the human genome: the domain structure for several PARPs is depicted in FIG. 3. However, the specific biological function and protein substrates of these PARPs are not fully characterized (Ame et al., *Bioessays* 26:882-893, 2004). The identification of the function and the substrates of each member of this family of proteins has been difficult to date.

PAR polymers are required for normal cell division and PARP knockouts in *Drosophila melanogaster* are embryonic lethal (Tulin et al., *Genes Dev.* 16:2108-2119, 2002). The concentration, length, and extent of PAR branching are regulated by a balance of activities of the PARPs and PAR glycohydrolase (PARG), a highly specific, processive endo- and exo-glycosidase (Hatakeyama et al., *J. Biol. Chem.* 261: 14902-14911, 1986). Poly-ADP-ribose polymers have generally been implicated for a role in several different human diseases including cancer, ischemic injury, inflammatory diseases, cardiovascular diseases, and neurodegenerative disorders.

We have discovered that several PARP proteins are localized to the nucleus and/or are required for cell cycle progression through mitosis. We have also discovered a role for several PARP proteins in the formation, nucleation, and disassembly of stress granules. Stress granules are distinct cellular structures that form in the cytosol upon exposure of a cell to stress conditions. Stress granules are composed of both proteins and RNA molecules. The RNA molecules present in stress granules are mRNA molecules stalled in translation pre-initiation complexes. Stress granules are typically 100 to 200 nM in size and are commonly associated with the endoplasmic reticulum. Stress granules have been implicated in

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several different disease states including cardiovascular disorders, inflammatory disorders, neurological disorders, and ischemic-reperfusion injury.

Methods and compositions for the treatment of stress granule-related disorders and cancer are presently desired.

SUMMARY OF THE INVENTION

The invention provides methods of treating or decreasing (e.g., by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%) the likelihood of developing a stress granule-related disorder in a subject requiring administering to a subject a therapeutically effective amount of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARP inhibitor(s), one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) poly-ADP ribose glycosylase (PARG) activators, and/or one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARP11 activators. The invention also provides methods for decreasing the number of stress granules present in a cell or in a population of cells requiring contacting the cell or population of cells with an effective amount of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARP inhibitor(s), one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARG activators, and/or one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARP11 activators. The invention also provides compositions and kits containing one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARP inhibitor(s), one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARG activator(s), and/or one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARP11 activators.

In each of the above of the above methods, compositions, and kits, the one or more PARP inhibitor(s) may selectively decrease (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, or even 100%) the expression (e.g., mRNA or protein) and/or activity of one or more (e.g., 1, 2, 3, 4, or 5) of PARP5a, PARP12, PARP13 isoform 1 (PARP13.1), PARP isoform 2 (PARP13.2), and PARP15. In different embodiments of the above aspects of the invention, the decrease in expression of one or more of PARP5a, PARP12, PARP13.1, PARP13.2, or PARP15 is a decrease in the level of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) nucleic acid(s) containing a nucleic acid sequence having at least 80% (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) sequence identity to PARP5a (SEQ ID NO: 8 or 9), PARP12 (SEQ ID NO: 18), PARP13.1 (SEQ ID NO: 19), PARP13.2 (SEQ ID NO: 20), or PARP15 (SEQ ID NO: 22 or 23), or a decrease in the level of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) polypeptides encoded by these nucleic acids. In different embodiments of the above aspects, the activity of the one or more of PARP5a, PARP12, PARP13.1, PARP13.2, or PARP15 is poly-ADP-ribosylation of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) target protein(s) (e.g., a protein localized in a stress granule, a polypeptide involved in the formation or disassembly of a stress granule, and/or a PARP protein) or formation and/or nucleation of a stress granule.

In each of the above aspects, the one or more PARG activators may selectively increase (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, or 100%) the expression (e.g., mRNA and/or protein) and/or one or more activities of PARG protein or poly-ADP-ribose glycohydrolase ARH3. In different embodiments of the above aspects of the invention, the increase in expression of PARG or ARH3 is an increase in the level of one or more (e.g., 1, 2, 3, 4, 5, or 6) nucleic acid(s) containing a nucleic acid sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARG (SEQ ID NO: 42) or ARH3 (SEQ ID NO: 41), or an increase in the level of one or more (e.g., 1, 2, 3, 4, 5, or 6) polypeptides encoded by

these nucleic acids. In different embodiments of the above aspects, the one or more (e.g., 1, 2, 3, 4, or 5) activities of PARG or ARH3 is hydrolysis of poly-ADP-ribose (e.g., poly-ADP-ribose attached to one or more (e.g., 1, 2, 3, 4, or 5) substrate protein(s), e.g., a protein localized in a stress granule, a polypeptide involved in the formation or disassembly of a stress granule, and/or a PARP protein), the prevention of the assembly of a stress granule, or disassembly of a stress granule.

In each of the above of the above methods, compositions, and kits, the one or more PARP11 activator (s) may selectively increase (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, or even 100%) the expression (e.g., mRNA and/or protein) and/or one or more (e.g., 1, 2, 3, 4, or 5) activities of PARP11. In different embodiments of the above aspects of the invention, the increase in expression of PARP11 is an increase in the level of one or more (e.g., 1, 2, 3, 4, or 5) nucleic acid(s) containing a nucleic acid sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARP11 (SEQ ID NO: 17), or an increase in the level of one or more (e.g., 1, 2, 3, 4, or 5) polypeptides encoded by these nucleic acids. In different embodiments of the above aspects, the one or more (e.g., 1, 2, 3, 4, or 5) activities of PARP11 is poly-ADP-ribosylation of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) target protein(s) (e.g., a protein localized in a stress granule, a polypeptide involved in the formation or disassembly of a stress granule, and/or a PARP protein), the prevention of the assembly of a stress granule, or the disassembly of a stress granule.

In each of the above aspects, the one or more PARP inhibitors may be an antibody or antibody fragment that selectively binds one or more (e.g., 1, 2, 3, 4, or 5) of PARP5a, PARP12, PARP13.1, PARP13.2, and PARP15; an RNA aptamer (e.g., one or more RNA aptamers containing the sequence of one of SEQ ID NOS: 40, 49, 99-113, and 122-129); or a small molecule. In each of the above aspects, the one or more PARG activators may be one or more (e.g., 1, 2, 3, 4, or 5) nucleic acid(s) containing a nucleic acid sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARG (SEQ ID NO: 42) or ARH3 (SEQ ID NO: 41). In each of the above aspects, the one or more PARP11 activators may be one or more (e.g., 1, 2, 3, 4, or 5) nucleic acid(s) containing a nucleic acid sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARP11 (SEQ ID NO: 17).

In each of the above methods of treatment, the one or more PARP inhibitor(s), one or more PARG activator(s), and/or one or more PARP11 activators may be administered once a day or one or more times a week (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 times a week), and/or administered parenterally (e.g., intravenous, intraarterial, subcutaneous, or intramuscular administration) or orally, and/or administered with one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) additional therapeutic agents (e.g., one or more (e.g., 1, 2, 3, 4, or 5) non-steroidal anti-inflammatory drug(s), one or more (e.g., 1, 2, 3, 4, or 5) immunosuppressive agent(s), one or more (e.g., 1, 2, 3, 4, or 5) calcineurin inhibitors, and/or one or more (e.g., 1, 2, 3, 4, or 5) analgesic(s)). In each of the above methods of treating a cell or cell population, the contacting may result in a reduction in the number of stress granules in a cell (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100% reduction in the number of stress granules in a cell or cell population compared to a control cell or cell population, e.g., a cell or cell population from a person having or diagnosed with a stress granule-related disorder).

The invention further provides methods of treating or decreasing (e.g., by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%) the likelihood of developing cancer in a subject requiring administering to a subject a therapeutically effective amount of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARP inhibitor(s). The invention also provides methods for decreasing (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or even 100%) proliferation of a cell or a population of cells requiring contacting the cell or population of cells with an effective amount of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARP inhibitor(s). The invention also provides compositions and kits containing one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARP inhibitor(s).

In each of these methods, compositions, and kits, the one or more PARP inhibitor(s) may selectively decrease (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, or even 100% decrease) the expression (e.g., mRNA and/or protein) and/or one or more (e.g., 1, 2, 3, 4, or 5) activities of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, or 8) of PARP1, PARP2, PARP5A, PARP5B, PARP7, PARP8, PARP14, and PARP16. In different embodiments of these aspects of the invention, the decrease in expression of the one or more of PARP1, PARP2, PARP5A, PARP5B, PARP7, PARP8, PARP14, and PARP16 is a decrease in the level of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) nucleic acid(s) containing a nucleic acid sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARP1 (SEQ ID NO: 1 or 2), PARP2 (SEQ ID NO: 3), PARP5A (SEQ ID NO: 8 or 9), PARP5B (SEQ ID NO: 10), PARP14 (SEQ ID NO: 21), or PARP16 (SEQ ID NO: 24), or a decrease in the level of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) polypeptides encoded by these nucleic acids. In different embodiments of these aspects of the invention, the one or more activities of the one or more of PARP1, PARP2, PARP5A, PARP5B, PARP7, PARP8, PARP14, or PARP16 is poly-ADP-ribosylation of one or more (e.g., 1, 2, 3, 4, or 5) target protein(s) (e.g., a protein localized in the nucleus or mitotic spindle during cytokinesis, and/or a PARP protein) or is required for progression through mitosis.

In each of these aspects, the one or more PARP inhibitors may be an antibody or antibody fragment that selectively binds one or more (e.g., 1, 2, 3, 4, 5, 6, 7, or 8) of PARP1, PARP2, PARP5A, PARP5B, PARP7, PARP8, PARP14, and PARP16; an RNA aptamer (e.g., an RNA aptamer containing the sequence of one or more of SEQ ID NOS: 43-46, 49, 50, 59-74, 114-121, and 130-136); or a small molecule. In each of these methods of treatment, the one or more PARP inhibitor(s) may be administered once a day or one or more times a week (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, or 14 times a week), and/or administered parenterally (e.g., intravenous, intraarterial, subcutaneous, or intramuscular administration) or orally, and/or administered with one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) additional therapeutic agents (e.g., one or more (e.g., 1, 2, 3, 4, or 5) chemotherapeutic agents, one or more (e.g., 1, 2, 3, 4, or 5) non-steroidal anti-inflammatory drug(s), one or more (e.g., 1, 2, 3, 4, or 5) immunosuppressive agent(s), one or more (e.g., 1, 2, 3, 4, or 5) calcineurin inhibitors, and/or one or more (e.g., 1, 2, 3, 4, or 5) analgesic(s)). In each of these methods of treating a cell or cell population, the contacting may result in a reduction in the rate of proliferation of a cell or cell population (e.g., by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100% reduction in the rate of proliferation of a cell or cell population compared to a control cell or cell population, e.g., a cancer cell or a cancer cell line).

The invention further provides methods for increasing (e.g., by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100%) the number of stress granules in a cell or cell population by contacting the cell or cell population with an effective amount of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARP activators, one or more (e.g., 1, 2, 3, 4, or 5) PARG inhibitors, and/or one or more (e.g., 1, 2, 3, 4, or 5) PARP11 inhibitors. In one embodiment of these methods, the one or more PARP activators selectively increase the expression (e.g., mRNA and/or protein) and/or one or more (e.g., 1, 2, 3, 4, or 5) activities of one or more (e.g., 1, 2, 3, 4, or 5) of PARP5A, PARP12, PARP13.1, PARP13.2, and PARP15. In different embodiments of these methods, the increase in expression is an increase (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100% increase) in the level of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) nucleic acid(s) containing a nucleic acid sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARP5A (SEQ ID NO: 8 or 9), PARP5b (SEQ ID NO: 10), PARP13.1 (SEQ ID NO: 19), PARP13.2 (SEQ ID NO: 20), or PARP15 (SEQ ID NO: 22 or 23), or an increase in the level of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) polypeptides encoded by these nucleic acids. In different embodiments of this method, the one or more activities of PARP5A, PARP12, PARP13.1, PARP13.2, and PARP15 is the poly-ADP-ribosylation of one or more (e.g., 1, 2, 3, 4, or 5) target protein(s) (e.g., a protein localized in a stress granule, a protein involved in the formation or disassembly of a stress granule, and/or a PARP protein) or the formation or nucleation of a stress granule.

In different embodiments of this method, the one or more PARG inhibitors may selectively decrease (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100%) the expression (e.g., mRNA and/or protein) and/or one or more (e.g., 1, 2, 3, 4, or 5) activities of PARG or ARH3. In additional embodiments of this method, the decrease in expression of PARG or ARH3 is a decrease in the level of one or more (e.g., 1, 2, 3, 4, 5, or 6) nucleic acid(s) containing a nucleic sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARG (SEQ ID NO: 42) or ARH3 (SEQ ID NO: 41), or a decrease in the levels of one or more (e.g., 1, 2, 3, 4, 5, or 6) polypeptides encoded by these nucleic acids. In additional embodiments of this method, the one or more activities of PARG or ARH3 is the hydrolysis of poly-ADP-ribose (e.g., poly-ADP-ribose that is covalently attached to one or more (e.g., 1, 2, 3, 4, or 5) substrate protein(s), e.g., a protein localized in a stress granule, a protein involved in the formation or disassembly of a stress granule, and/or a PARP protein), the prevention of assembly of a stress granule protein, or disassembly of a stress granule.

In an additional embodiment of this method, the one or more PARP11 inhibitors may selectively decrease (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100%) the expression (e.g., mRNA and/or protein) and/or one or more activities of PARP11. In different embodiments of this method, the decrease (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100%) in expression of PARP11 is a decrease in the level of one or more (e.g., 1, 2, 3, 4, or 5) nucleic acid(s) containing a nucleic acid having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARP11 (SEQ ID NO: 17), or a decrease in the level of one or more (e.g., 1, 2, 3, 4, or 5) polypeptides encoded by these nucleic acids. In different embodiments of this method, the one or more activities of PARP11 is poly-ADP-ribosylation of one or more (e.g., 1, 2, 3, 4, or 5) target protein(s) (e.g., a protein

localized in a stress granule, a protein involved in the formation or disassembly of a stress granule, and/or a PARP protein), the prevention of the assembly of a stress granule, or disassembly of a stress granule.

In additional embodiments of this method, the one or more PARP activators is one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) nucleic acid(s) comprising a nucleic acid sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARP5A (SEQ ID NO: 8 or 9), PARP5B (SEQ ID NO: 10), PARP13.1 (SEQ ID NO: 19), PARP13.2 (SEQ ID NO: 20), or PARP15 (SEQ ID NO: 22 or 23). In additional embodiments of this method, the one or more PARG inhibitors is an antibody or antibody fragment that selectively binds to PARG or ARH3; an RNA aptamer (e.g., a nucleic acid sequence that contains one or more of SEQ ID NOS: 34-37); or a small molecule. In additional embodiments of this method, the one or more PARP11 inhibitors may be an antibody or an antibody fragment that selectively binds to PARP11; an RNA aptamer (e.g., a nucleic acid sequence that contains one or more of SEQ ID NOS: 91-98); or a small molecule.

In additional embodiments of this method, the contacting results in at least a 10% (e.g., at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100%) reduction in the number of stress granules present in the cell or the population of cells compared to a control cell or population of cells (e.g., a cell or population of cells untreated with a PARP activator, a PARG inhibitor, or a PARP11 inhibitor, e.g., a cell from a subject having or diagnosed with a stress-granule disorder). In additional embodiments of this method, the cell or population of cells is a mammalian cell(s) or a plant cell(s).

The invention also provides methods of increasing (e.g., by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100%) the proliferation of a cell or a population of cells requiring contacting the cell or population of cells with an effective amount of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARP activators. In different embodiments of this method, the one or more PARP activators selectively increase (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100%) the expression (e.g., mRNA and/or protein) and/or one or more activities (e.g., 1, 2, 3, 4, or 5) of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, or 8) of PARP1, PARP2, PARP5A, PARP5B, PARP7, PARP8, PARP14, and PARP16. In additional embodiments of this method, the increase in expression of one or more of PARP1, PARP2, PARP5A, PARP5B, PARP7, PARP8, PARP14, or PARP16 is an increase in the level of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) nucleic acid(s) comprising a nucleic acid sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARP1 (SEQ ID NO: 1 or 2), PARP2 (SEQ ID NO: 3), PARP5A (SEQ ID NO: 8 or 9), PARP5B (SEQ ID NO: 10), PARP14 (SEQ ID NO: 21), or PARP16 (SEQ ID NO: 24), or an increase in the level of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) polypeptides encoded by these nucleic acids. In additional embodiments of this method, the one or more activities of said one or more of PARP1, PARP2, PARP5A, PARP5B, PARP7, PARP8, PARP14, or PARP16 is poly-ADP-ribosylation of one or more (e.g., 1, 2, 3, 4, or 5) target protein(s) (e.g., a protein localized in the nucleus or mitotic spindle during cytokinesis, and/or a PARP protein) or is required for progression through mitosis.

In additional embodiments of this method, the one or more PARP activators is one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) nucleic acid(s) containing a nucleic acid sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARP1 (SEQ ID

NO: 1 or 2), PARP2 (SEQ ID NO: 3), PARP5A (SEQ ID NO: 8 or 9), PARP5B (SEQ ID NO: 10), PARP14 (SEQ ID NO: 21), or PARP16 (SEQ ID NO: 24). In different embodiments of this method, the cell or cell population is a plant cell(s) or mammalian cell(s). In different embodiments of this method, the contacting results in at least a 10% increase (e.g., at least a 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100% increase) in the rate of proliferation of the cell or population of cells compared to a control cell or cell population (e.g., a cell or cell population not treated with a PARP activator).

The invention further provides methods for identifying a candidate agent for treating or decreasing (e.g., by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%) the likelihood of developing a stress granule-related disorder requiring the steps of: providing one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) PARP, PARG, and/or ARH3 proteins, and/or PARP, PARG, and/or ARH3 fusion proteins encoded by a nucleic acid containing a nucleic acid sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARP5A (SEQ ID NO: 8 or 9), PARP12 (SEQ ID NO: 18), PARP13.1 (SEQ ID NO: 19), PARP13.2 (SEQ ID NO: 20), PARP15 (SEQ ID NO: 22 or 23), PARG (SEQ ID NO: 42), or ARH3 (SEQ ID NO: 41); contacting the one or more PARP, PARG, and/or ARH3 proteins, and/or PARP, PARG, and/or ARH3 fusion proteins with the agent and a labeled nicotinamide adenine dinucleotide (NAD⁺) substrate; and measuring one or more (e.g., 1, 2, 3, 4, or 5) activities of the one or more PARP, PARG, and/or ARH3 proteins, and/or PARP, PARG, and/or ARH3 fusion proteins, or the specific binding of the agent to said one or more PARP proteins and/or PARP fusion proteins; wherein an agent that decreases (e.g., by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%) the one or more activities and/or specifically binds to the one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16) PARP proteins and/or PARP fusion proteins, and/or increases the activity of the one or more (e.g., 1, 2, 3, 4, 5, 6, 7, or 8) PARG and/or ARH3 proteins, and/or PARG and/or ARH3 fusion proteins is identified as a candidate agent for treating or decreasing the likelihood of developing a stress granule-related disorder.

The invention further provides methods for identifying a candidate agent for treating or decreasing (e.g., by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%) the likelihood of developing cancer requiring the steps of providing one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16) PARP protein(s) and/or PARP fusion protein(s) encoded by a nucleic acid containing a nucleic acid sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARP1 (SEQ ID NO: 1 or 2), PARP2 (SEQ ID NO: 3), PARP5A (SEQ ID NO: 8 or 9), PARP5B (SEQ ID NO: 10), PARP7 (SEQ ID NO: 12), PARP8 (SEQ ID NO: 13), PARP14 (SEQ ID NO: 21), or PARP16 (SEQ ID NO: 24); contacting the one or more PARP protein(s) and/or PARP fusion protein(s) with the agent and a labeled NAD⁺ substrate; and measuring the one or more (e.g., 1, 2, 3, 4, or 5) activities of the one or more PARP protein(s) and/or PARP fusion protein(s), and/or the specific binding of the agent to the one or more PARP protein(s) and/or PARP fusion protein(s); wherein an agent that decreases (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%) the one or more activities and/or specifically binds to the one or more PARP protein(s) and/or fusion protein(s) is identified as a candidate agent for treating or reducing the likelihood of developing cancer.

In different embodiments of the screening methods, the labeled NAD⁺ substrate is labeled with a radioisotope

(e.g., ³²P) or fluorophore, or is biotinylated. In different embodiments of the screening methods, the one or more PARP, PARG, and/or ARH3 protein(s) and/or PARP, PARG, and/or ARH3 fusion protein(s) is purified. In another embodiment of the screening methods, the one or more PARP, PARG, and/or ARH3 protein(s) and/or PARP, PARG, and/or ARH3 fusion protein(s) is present in a cell lysate. In different embodiments of the screening methods, the agent is a small molecule (e.g., a small molecule from a chemical library), a polypeptide or peptide fragment (e.g., a polypeptide or peptide fragment present in a cellular lysate), or a nucleic acid. In additional embodiments of the above screening methods, the one or more PARP, PARG, and/or ARH3 protein(s) and/or PARP, PARG, and/or ARH3 fusion protein(s) is attached to a substrate (e.g., a magnetic bead) or a solid surface. In additional embodiments of the above screening methods, the method is performed in a multi-well plate.

The invention further provides methods for determining the propensity of a subject to develop a stress granule-related disorder by determining the expression and/or one or more (e.g., 1, 2, 3, 4, or 5) activities of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, or 8) of PARP5A, PARP11, PARP12, PARP13.1, PARP13.2, PARP15, PARG, and ARH3 in a subject, wherein an increase (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%) in the expression (e.g., mRNA and/or protein) and/or one or more (e.g., 1, 2, 3, 4, or 5) activities of one or more (e.g., 1, 2, 3, 4, or 5) of PARP5A, PARP12, PARP13.1, PARP13.2, and PARP15, and/or a decrease (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100%) in the expression (e.g., mRNA and/or protein) or one or more (e.g., 1, 2, 3, 4, or 5) activities of one or more (e.g., 1, 2, or 3) of PARP11, PARG, or ARH3 indicates an increased propensity to develop a stress granule-related disorder. In an additional embodiment of this method, the expression is the level of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) nucleic acid(s) containing a nucleic acid sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARP5A (SEQ ID NO: 8 or 9), PARP5B (SEQ ID NO: 10), PARP11 (SEQ ID NO: 17), PARP13.1 (SEQ ID NO: 19), PARP13.2 (SEQ ID NO: 20), PARP15 (SEQ ID NO: 20 or 23), PARG (SEQ ID NO: 42), or ARH3 (SEQ ID NO: 41), or the level of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) polypeptides encoded by these nucleic acids.

The invention also provides a method for determining the propensity of a subject to develop cancer comprising determining the expression (e.g., mRNA and/or protein) and/or one or more (e.g., 1, 2, 3, 4, or 5) activities of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, or 8) of PARP1, PARP2, PARP5A, PARP5B, PARP7, PARP8, PARP14, and PARP16 in a subject, wherein an increase in the expression and/or one or more activities of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, or 8) of PARP1, PARP2, PARP5A, PARP5B, PARP7, PARP8, PARP14, and PARP16 indicates an increased propensity to develop cancer. In an additional embodiment of this method, the expression is the level of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) nucleic acid(s) containing a nucleic acid sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARP1 (SEQ ID NO: 1 or 2), PARP2 (SEQ ID NO: 3), PARP5A (SEQ ID NO: 8 or 9), PARP5B (SEQ ID NO: 10), PARP7 (SEQ ID NO: 12), PARP8 (SEQ ID NO: 13), PARP14 (SEQ ID NO: 21), or PARP16 (SEQ ID NO: 24), or the level of one or more polypeptides (e.g., 1, 2, 3, 4, 5, 6, 7, or 8) encoded by these nucleic acids.

In any of the above screening methods, the expression of the one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) nucleic

acid(s) is determined using reverse transcriptase polymerase chain reaction (RT-PCR). In additional embodiments of the above screening methods, the expression of the one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) polypeptide(s) is determined using an antibody-based technique (e.g., immunoblotting or an enzyme-linked immunosorbent assay (ELISA)).

In all of the above aspects, a stress granule-related disorder may be a cardiovascular disorder (e.g., an aneurysm, angina, atherosclerosis, stroke, cerebrovascular disease, congestive heart failure, coronary artery disease, myocardial disease, peripheral vascular disease, granulomatous myocarditis, chronic myocarditis, myocardial infarction, and primary hypertrophic cardiomyopathy), an inflammatory disorder (e.g., an autoimmune disease, asthma, an allergic intraocular inflammatory disease, arthritis, atopic dermatitis, atopic eczema, cirrhosis, Crohn's disease, ulcerative colitis, diabetes, hemolytic anemia, inflammatory dermatosis, an inflammatory bowel disorder, systemic lupus erythematosus, psoriasis, rheumatoid arthritis, Wegener's granulomatosis, Hashimoto's thyroiditis, chronic pancreatitis, and reactive lymphoid hyperplasia), a neurological disorder (e.g., multiple sclerosis, Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, retinosa pigmentosa, macular degeneration, traumatic brain injury, stroke, and peripheral neuropathy), or ischemic-reperfusion injury. In different embodiments of all the above aspects, the treating may result in a reduction (e.g., at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100% reduction) in one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) symptoms of a stress granule-related disorder or a reduction (e.g., at least a 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100% reduction) in the likelihood of developing a stress granule-related disorder.

In all the above aspects, the cancer may be colon adenocarcinoma, esophagus adenocarcinoma, liver hepatocellular carcinoma, squamous cell carcinoma, pancreas adenocarcinoma, islet cell tumor, rectum adenocarcinoma, gastrointestinal stromal tumor, stomach adenocarcinoma, adrenal cortical carcinoma, follicular carcinoma, papillary carcinoma, breast cancer, ductal carcinoma, lobular carcinoma, intraductal carcinoma, mucinous carcinoma, phyllodes tumor, Ewing's sarcoma, ovarian adenocarcinoma, endometrium adenocarcinoma, granulosa cell tumor, mucinous cystadenocarcinoma, cervix adenocarcinoma, vulva squamous cell carcinoma, basal cell carcinoma, prostate adenocarcinoma, giant cell tumor of bone, bone osteosarcoma, larynx carcinoma, lung adenocarcinoma, kidney carcinoma, urinary bladder carcinoma, Wilms' tumor, lymphoma, and non-Hodgkin's lymphoma. In different embodiments of all the above aspects, said treating may result in a reduction (e.g., at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100% reduction) in one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) symptoms of a cancer or a reduction (e.g., at least a 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100% reduction) in the likelihood of developing a cancer.

In all the above aspects of the invention, the cell or population of cells is an epithelial cell, a fibroblast, a kidney cell, a muscle cell, a neuron, a hepatocyte, an oocyte, a sperm, a lymphocyte, or a macrophage.

In all the above aspects of the invention, the compositions may be formulated for parenteral or oral administration.

By the term "ARH3" or "poly-ADP-ribose glycohydrolase ARH3" is meant a nucleic acid having containing a sequence at least 80% identical (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100% identical) to the sequence of SEQ ID NO: 41, or one or more polypeptides encoded by these nucleic acids.

By the term "ARH3 fusion protein" or "poly-ADP-ribose glycohydrolase ARH3 fusion protein" is meant a polypeptide containing a polypeptide tag and a sequence having at least 80% identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100% identity) to a protein encoded by ARH3 (SEQ ID NO: 41). The polypeptide tag of an ARH3 fusion protein may be located at the N- and/or C-terminus of the protein. The polypeptide tag may contain one or more of a fluorescent protein (e.g., a green fluorescence protein), a peptide epitope recognized by specific antibodies, a protein that is bound by a partner binding protein with high affinity (e.g., biotin and streptavidin), a His₆-tag, or one or more (e.g., 1, 2, 3, 4, 5, 6, or 7) protease recognition sequence(s) (e.g., one or more of a TEV protease or Factor Xa protease recognition sequence). ARH3 fusion proteins may be purified using antibodies specific for the polypeptide tag. For example, antibodies specific for the polypeptide tag or proteins that bind specifically to the protein sequence in the polypeptide tag may bound to a bead (e.g., a magnetic bead) or polymer surface in order to allow for the purification of the ARH3 fusion protein. An ARH3 fusion protein may also be purified and subsequently treated with one or more (e.g., 1, 2, or 3) protease(s) to remove the polypeptide tag from the ARH3 fusion protein. An ARH3 fusion protein preferably has the same cellular localization and biological activity as the wild-type ARH3 protein. Methods for the generation and purification of ARH3 fusion proteins are described herein.

By the term "biotinylated" is meant the covalent attachment of a biotin molecule to a small molecule, surface, or protein. A biotin molecule may be attached to a small molecule, surface, or protein using methods known in the art including, but not limited to, attachment to primary amines (e.g., epsilon-amines and N-terminal α -amines of a protein), as well as attachment at a sulfhydryl group, and a carboxyl group. Small molecules (e.g., NAD⁺) and proteins (e.g., one or more of the PARP fusion proteins described herein) may be biotinylated. Biotinylated NAD⁺ is available from a number of commercial sources including R & D Systems, Gentaur, and Trevigen (e.g., 6-biotin-17-NAD). Biotinylated small molecules and substrates may be specifically bound and/or purified using streptavidin, a protein that has a high affinity for biotin ($K_a \sim 10^{13} \text{ M}^{-1}$), or surfaces covalently attached to streptavidin (e.g., streptavidin-coated beads).

By the term "cancer" is meant a disease of uncontrolled or misregulated cell proliferation or cell division. Non-limiting examples of cancer include colon adenocarcinoma, esophagus adenocarcinoma, liver hepatocellular carcinoma, squamous cell carcinoma, pancreas adenocarcinoma, islet cell tumor, rectum adenocarcinoma, gastrointestinal stromal tumor, stomach adenocarcinoma, adrenal cortical carcinoma, follicular carcinoma, papillary carcinoma, breast cancer, ductal carcinoma, lobular carcinoma, intraductal carcinoma, mucinous carcinoma, phyllodes tumor, Ewing's sarcoma, ovarian adenocarcinoma, endometrium adenocarcinoma, granulosa cell tumor, mucinous cystadenocarcinoma, cervix adenocarcinoma, vulva squamous cell carcinoma, basal cell carcinoma, prostate adenocarcinoma, giant cell tumor of bone, bone osteosarcoma, larynx carcinoma, lung adenocarcinoma, kidney carcinoma, urinary bladder carcinoma, Wilms' tumor, lymphoma, and non-Hodgkin's lymphoma.

By the term "cell lysate" is meant the contents of the cell once the plasma membrane has been disrupted or permeabilized. Cell lysate also includes the contents of the intracellular organelles (e.g., endoplasmic reticulum, nucleus, mitochondria, chloroplasts, Golgi apparatus, and lysosome) upon disruption of their respective membranes. Cell lysate contains an unpurified mixture of proteins, small molecule metabolites,

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and nucleic acids (e.g., DNA and RNA). Cell lysate may be prepared from any type of cell, e.g., a mammalian cell (e.g., human, mouse, rat, and monkey cell), a bacterial cell, fungal cell, and a yeast cell. Cell lysate may be obtained by any methods known in the art including physical disruption (e.g., sonication, homogenization, or freeze/thaw procedures) or chemical disruption (e.g., treatment with a detergent (e.g., Triton-X-100 and NP-40)). Cell lysate may be prepared from a cell expressing one or more of the nucleic acid(s) that encode one or more PARP, PARG, and/or ARH3 proteins and/or one or more PARP, PARG, and/or ARH3 fusion protein(s). Cell lysate may also be prepared from a cell arrested in a specific stage of the cell cycle (e.g., mitosis or S-phase) or may be prepared from asynchronous cells.

By the term “constitutive promoter” is meant a promoter that is placed 5' relative to a nucleic acid sequence encoding a protein, wherein the promoter regulates the consistent expression of a nucleic acid encoding a protein. The sequence of the constitutive promoter may be directly (no extraneous nucleotides) 5' to the first nucleotide of the sequence encoding the protein (e.g., a PARP, PARG, and/or ARH3 protein and/or a PARP, PARG, and/or ARH3 fusion protein as described herein) or may be between 1-20 nucleotides, 1-100 nucleotides, 10-260 nucleotides, 100-700 nucleotides, or 100 to 2,000 nucleotides from the first nucleotide of the sequence encoding the protein. Examples of constitute promoters include, but are not limited to, bacterial promoters (e.g., *E. coli* σ^{70} , σ^S , σ^{32} , or σ^{54} promoters; *B. subtilis* σ^A or σ^B promoters; T7 RNA polymerase-based promoters; and bacteriophage SP6 promoter), yeast promoters (e.g., pCyc, pAdh, pSte5, ADH1, cyc100, cyc70, cyc43, cyc28, cyc16, pPGK1, pCYC, GPD (TDH3), and CLB1 promoters), and mammalian promoters (e.g., cytomegalovirus immediate early gene-based promoters, SV40 early promoter, and Rous sarcoma virus promoter). A constitutive promoter may be used to mediate the expression of a nucleic acid (e.g., one or more nucleic acids encoding a PARP, PARG, and/or ARH3 protein, and/or a PARP, PARG, and/or ARH3 fusion protein as described herein) in a transgenic mammalian, bacterial, or yeast cell.

By the term “disassembly” of a stress granule is meant the process of deconstructing or dissolution of one or more stress granules in a cell or cell population. The disassembly of a stress granule may take place by the catalytic activity of one or more proteins (e.g., one or more of a PARG, ARH3, and/or PARP11, and/or a PARG, ARH3, and/or PARP11 fusion protein). The disassembly of a stress granule in a cell or cell population may occur following the removal of a stress condition (e.g., washout of sodium arsenite or pateamine A from the culture medium).

By the term “effective amount” or “therapeutically effective amount” is meant the amount of the agent administered to a subject, to a cell, or to a cell population that elicits a specific desirable effect. For example, the amount of an agent (e.g., one or more PARP inhibitor(s), one or more PARG activator(s), and/or one or more PARP11 activator(s)) that decreases the number (e.g., prevents 1, 2, 3, 4, or 5 symptoms from occurring) or severity (e.g., decrease of at least 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% in the severity) of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) symptoms of a stress granule-related disorder or the amount of an agent (e.g., one or more PARP inhibitor(s)) that decreases the number or severity of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) symptoms of cancer. The effective amount or therapeutically effective amount of an agent may be determined by a skilled artisan using methods known in the art and the methods described herein.

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By “formation” of a stress granule is meant a series of events that lead to the appearance of distinct protein- and RNA-containing stress granules in the cytoplasm. The formation of a stress granule in a cell may be accelerated by exposure to stress conditions, including, but not limited to, chemical stress (e.g., sodium arsenite and pateamine A). By “nucleation” of a stress granule is meant one of the initial steps or initial rate-limiting steps in the formation of a stress granule in a cell. Examples of proteins involved in the formation or nucleation of stress granules include, without limitation, PARP 5A, PARP12, PARP13.1, PARP13.2, and PARP 15.

By “labeled nicotinamide adenine dinucleotide” or “labeled NAD⁺” is meant a molecule of nicotinamide adenine dinucleotide (NAD⁺) that is covalently labeled with a fluorescent molecule, a colorimetric molecule, or a molecule that is recognized by a specific partner protein (e.g., biotinylation), or labeled with a radioisotope. One example of a labeled NAD⁺ is biotinylated NAD⁺ (e.g., 6-biotin-14-NAD). Examples of radiolabeled NAD⁺ include, but are not limited to, ¹⁴C-adenine-NAD⁺, ³²P-NAD⁺, and ³H-NAD⁺. Additional examples of labeled NAD⁺ are known in the art.

By the term “short RNA or DNA aptamer” is meant a short sequence of DNA or RNA nucleotides that binds to a specific target molecule (e.g., a protein or a target RNA or DNA molecule). A DNA or RNA aptamer that specifically binds to its target molecule (e.g., one or more (e.g., 1, 2, 3, 4, or 5) of the nucleic acids encoding a PARP, PARG, and/or ARH3 protein, and/or a PARP, PARG, and/or ARH3 fusion protein (as described herein) may decrease (e.g., by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%) or increase (e.g., by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%) one or more (e.g., 1, 2, 3, 4, or 5) activities or expression (e.g., mRNA or protein level) of the respective target molecule. For example, a specific DNA or RNA aptamer may bind to one or more of the above-described PARP proteins or PARP fusion proteins and increase or decrease the poly-ADP ribosylation activity of the protein, the amount of poly-ADP ribose attached to the protein, or the levels of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARP proteins or PARP fusion proteins. The specific DNA or RNA aptamer may also bind to one or more nucleic acids (e.g., DNA or RNA) that encode a specific PARP, PARG, and/or ARH3 protein (e.g., a nucleic acid that encodes a protein having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARP1 (SEQ ID NO: 1 or 2), PARP2 (SEQ ID NO: 3), PARP3 (SEQ ID NO: 4), PARP3.2 (SEQ ID NO: 5), PARP3.3 (SEQ ID NO: 6), PARP4 (SEQ ID NO: 7), PARP5A (SEQ ID NO: 8 or 9), PARP5B (SEQ ID NO: 10), PARP6 (SEQ ID NO: 11), PARP7 (SEQ ID NO: 12), PARP8 (SEQ ID NO: 13), PARP9 (SEQ ID NO: 14), PARP10 (SEQ ID NO: 15), PARP10.2 (SEQ ID NO: 16), PARP11 (SEQ ID NO: 17), PARP12 (SEQ ID NO: 18), PARP13.1 (SEQ ID NO: 19), PARP13.2 (SEQ ID NO: 20), PARP14 (SEQ ID NO: 21), PARP15.1 (SEQ ID NO: 22), PARP15.2 (SEQ ID NO: 23), PARP16 (SEQ ID NO: 24), PARG (SEQ ID NO: 42), and ARH3 (SEQ ID NO: 41)), and mediate an increase or decrease in the expression (e.g., protein and/or mRNA level) of the PARP, PARG, or ARH3. A specific example of an RNA aptamer is an inhibitory RNA (RNAi) molecule. Methods for the design of RNAi molecules are known in the art. Examples of specific RNAi molecules that may be used to decrease the expression of a PARP, PARG, and/or ARH3 protein, and/or PARP, PARG, and/or ARH3 fusion protein are described herein.

By the term “fluorescent protein” is meant a protein that absorbs light of a specific wavelength (e.g., absorption wave-

length) and emits light with a longer wavelength (e.g., emission wavelength). The term fluorescent protein encompasses natural fluorescent proteins (i.e., the natural form of the fluorescent protein without any genetic manipulations) and genetically mutated fluorescent proteins (e.g., fluorescent proteins engineered to change the identity of one or more amino acid residues). Several different examples of fluorescent proteins are known in the art, including, but limited to, green fluorescent proteins (e.g., GFP, Emerald, Superfolder GFP, Azami Green, mWasabi, TagGFP, TurboGFP, AcGFP, ZsGreen, T-Sapphire, and T-Sapphire), blue fluorescent proteins (e.g., EBFP, EBFP2, Azurite, mTagBFP), cyan fluorescent proteins (e.g., ECFP, mECFP, Cerulean, CyPet, AmCyan1, Midori-Ishi Cyan, TagCFP, mTFP1 (Teal)), yellow fluorescent proteins (e.g., EYFP, Topaz, Venus, mCitrine, YPet, TanYFP, PhiYFP, ZsYellow1, and mBanana), orange fluorescent proteins (e.g., Kurabira Orange, Kurabira Orange2, mOrange, mOrange2, dTomato, dTomato-Tandem, TagRFP, TagRFP-T, DsRed, DsRed2, DsRed-Express (T1), DsRed-Monomer, and mTangerine), and red fluorescent proteins (e.g., mRuby, mApple, mStrawberry, AsRed2, mRFP1, JRed, mCherry, HcRed1, mRaspberry, dKeima-Tandem, HcRed-Tandem, mPlum, and AQ143). Fluorescent proteins may be attached to the N- and/or C-terminus of a target protein (e.g., one or more of the PARP, PARG, and/or ARH3 fusion proteins described herein). Fusion proteins tagged with a fluorescent protein (e.g., one or more of the PARP, PARG, and/or ARH3 fusion proteins described herein) may be analyzed using fluorescence-based techniques known in the art (e.g., fluorescence microscopy, fluorescence plate readers, fluorescence-assisted cell sorting, and use of a second antibody specific for the fluorescent protein).

By the term "inducible promoter" is meant a promoter that is placed 5' relative to a nucleic acid sequence encoding a protein, wherein the promoter induces (or represses) the expression of a nucleic acid upon addition (or removal) of a specific molecule or protein. The sequence of the inducible promoter may be directly (no extraneous nucleotides) 5' to the first nucleotide of the sequence encoding the protein (e.g., a PARP fusion protein as described herein) or may be between 1-20 nucleotides, 1-100 nucleotides, 10-260 nucleotides, 100-700 nucleotides, or 100 to 2,000 nucleotides from the first nucleotide of the sequence encoding the protein. Examples of inducible promoters include, but are not limited to alcohol dehydrogenase I gene promoters, tetracycline-responsive promoter systems, glucocorticoid receptor promoters, estrogen receptor promoter, ecdysone receptor promoters, metallothionein-based promoters, and T7-polymerase based promoters. An inducible promoter may be used to regulate the expression of a nucleic acid (e.g., one or more nucleic acids encoding a PARP, PARG, and/or ARH3 protein and/or PARP, PARG, and/or ARH3 fusion protein as described herein) in a transgenic mammalian, bacterial, or yeast cell.

By the term "nuclear lysate" is meant the contents of a nucleus upon disruption of the nuclear membrane. Nuclear lysate contains an unpurified mixture of proteins, small molecule metabolites, and nucleic acids (e.g., DNA and RNA). Nuclear lysate may be prepared from any type of nucleated cell, e.g., a mammalian cell (e.g. human, mouse, rat, and monkey cell), a fungal cell, a yeast cell, or a plant cell. Nuclear lysate may be obtained by any methods known in the art including stepped lysis using two different concentrations of detergents (e.g., NP-40) or a combination of physical treatment to rupture the plasma membrane and chemical treatment to rupture the nuclear membrane. Nuclear lysate may be prepared from a cell expressing one or more of the nucleic

acid(s) of the invention that encode a one or more PARP, PARG, or ARH3 proteins or PARP, PARG, or ARH3 fusion protein(s).

By "PAR" or "poly-ADP ribose" is meant a chain of two or more ADP-ribose molecules. The two or more molecules of ADP-ribose making up PAR may occur in a single linear chain or as a branched chain with one or more branches (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 branches). Poly-ADP ribose may be attached to a specific substrate (e.g., protein, lipid, DNA, RNA, or small molecule) by the activity of one or more PARP proteins or PARP fusion proteins (e.g., one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, or 22) of PARP1, PARP2, PARP3, PARP3.2, PARP3.3, PARP4, PARP5A, PARP5B, PARP6, PARP7, PARP8, PARP9, PARP10, PARP11, PARP12, PARP13.1, PARP13.2, PARP14, PARP15.1, PARP15.2, and PARP16, or one or more of their respective fusion proteins) or removed by the activity of one or more PARG protein, PARG fusion protein, ARH3 protein, or ARH3 fusion protein (e.g., PARG protein or ARH3). Attachment of poly-ADP-ribose to a substrate protein may affect the biological activity of the substrate protein, localization of the protein, or the identity and number of proteins that bind to the target substrate (e.g., protein). PARP proteins may also be modified by the covalent attachment of poly-ADP-ribose. The addition of poly-ADP ribose to a PARP protein may occur by "auto-modification" or "auto-modulation" (i.e., a specific PARP catalyzes the attachment of poly-ADP ribose to itself) or may occur by the activity of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) other PARP proteins.

By the term "poly-ADP-ribose glycolase" or "PARG" is meant any enzyme that has the ability to remove an ADP-ribose attached to a substrate (e.g., a protein, RNA molecule, DNA molecule, or lipid) or to remove one or more ADP-ribose molecules from a pre-existing poly-ADP-ribose molecule covalently attached to a substrate (e.g., a protein, RNA molecule, DNA molecule, or lipid). For example, a PARG may be one or more nucleic acids containing a sequence having at least 80% identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARG (SEQ ID NO: 42) or ARH3 (SEQ ID NO: 41), or one or more polypeptides encoded by these nucleic acids. A PARG may have additional biological activities, such as decreasing (e.g., by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or even 100%) the formation or rate of formation of a stress granule in a cell or increasing the rate of disassembly of a stress granule. The term PARG also includes the isoforms of PARG proteins described in Meyer-Ficca et al., *Exp. Cell. Res.* 297(2):521-532, 2004.

By the term "PARG protein" or "poly-ADP-ribose glycolase protein" is meant a polypeptide encoded by a nucleic acid containing a sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100% identical) to the sequence of SEQ ID NO: 42.

By the term "PARG fusion protein" or "poly-ADP-ribose glycolase fusion protein" is meant a polypeptide containing a polypeptide tag and a sequence encoded by a nucleic acid containing a sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100% identity) to PARG (SEQ ID NO: 42). The polypeptide tag of a PARG fusion protein may be located at the N- and/or C-terminus of the protein. The polypeptide tag may contain one or more of a fluorescent protein (e.g., a green fluorescence protein), a peptide epitope recognized by specific antibodies, a protein that is bound by a partner binding protein with high affinity (e.g., biotin and streptavidin), a His₆-tag, or

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one or more (e.g., 1, 2, 3, 4, 5, 6, or 7) protease recognition sequence(s) (e.g., one or more of a TEV protease or Factor Xa protease recognition sequence). PARG fusion proteins may be purified using antibodies specific for the polypeptide tag. For example, antibodies specific for the polypeptide tag or proteins that bind specifically to the protein sequence in the polypeptide tag may bound to a bead (e.g., a magnetic bead) or polymer surface in order to allow for the purification of the PARG fusion protein. A PARG fusion protein may also be purified and subsequently treated with one or more (e.g., 1, 2, or 3) protease(s) to remove the polypeptide tag from the PARG fusion protein. A PARG fusion protein preferably has the same cellular localization and biological activity as the wild-type PARG protein. Methods for the generation and purification of PARG fusion proteins are described herein.

By the term “poly-ADP-ribose glycolase activator” or “PARG activator” is meant an agent that increases (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100%) the expression (e.g., protein and/or mRNA level) or one or more (e.g., 1, 2, 3, 4, or 5) biological activities of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARG proteins. For example, a PARG activator may increase the levels of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) nucleic acids containing a nucleic acid sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARG (SEQ ID NO: 42) or ARH3 (SEQ ID NO: 41), or increase the level of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) polypeptides encoded by these nucleic acids. A PARG activator may increase one or more of the biological activities of a PARG including the ability to remove a ADP-ribose attached to a substrate (e.g., a protein, RNA molecule, DNA molecule, lipid, or small molecule), the ability to remove one or more ADP-ribose molecules from a pre-existing poly-ADP-ribose molecule covalently attached to one or more substrate(s) (e.g., a protein, RNA molecule, DNA molecule, lipid, or small molecule), the ability to decrease or prevent the formation or the rate of formation of a stress granule in a cell, or the ability to increase the rate of disassembly of a stress granule. Non-limiting examples of PARG activators include one or more nucleic acids containing a nucleic acid having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARG (SEQ ID NO: 42) or ARH3 (SEQ ID NO: 41).

By the term “poly-ADP-ribose glycolase inhibitor” or “PARG inhibitor” is meant an agent that decreases (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100%) the expression (e.g., protein and/or mRNA level) or one or more (e.g., 1, 2, 3, 4, or 5) biological activities of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARGs. For example, a PARG inhibitor may decrease the levels of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) nucleic acids containing a nucleic acid sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARG (SEQ ID NO: 42) or ARH3 (SEQ ID NO: 41), or decrease the level of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) polypeptides encoded by these nucleic acids. A PARG activator may decrease one or more (e.g., 1, 2, 3, 4, or 5) of the biological activities of a PARG including, but not limited to, the ability to remove a ADP-ribose attached to one or more substrate(s) (e.g., a protein, RNA molecule, DNA molecule, lipid, or small molecule), the ability to remove one or more ADP-ribose molecules from a pre-existing poly-ADP-ribose molecule covalently attached to a substrate (e.g., a protein, RNA molecule, DNA molecule, lipid, or small molecule), the ability to decrease or prevent the formation or the rate of formation of

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a stress granule in a cell, or the ability to increase the rate of disassembly of a stress granule. Non-limiting examples of PARG inhibitors include antibodies or antibody fragments that specifically bind to PARG protein, ARH3 protein, PARG fusion protein, or ARH3 fusion protein; RNAi molecules (e.g., a nucleic acid sequence that contains the sequence of one of SEQ ID NOS: 34-37), or small molecules.

By the term “peptide fragment” is meant a protein having at least 2 amino acids (e.g., at least 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acids), but having fewer amino acids than the wild-type protein. Non-limiting examples of peptide fragments have between 2 to 250 amino acids, 5 to 200 amino acids, between 5 to 150 amino acids, or between 5 to 100 amino acids. A peptide fragment may also represent a protein that has been processed to remove one or more (e.g., 1, 2, or 3) post-translational targeting sequences (e.g., nuclear localization sequence, ER signal peptide, mitochondrial targeting signal, nuclear export sequence, or N-terminal secretion sequence).

By “poly-ADP ribose polymerase nucleic acid” or “PARP nucleic acid” is meant any nucleic acid containing a sequence that has at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100% sequence identity) to one or more of PARP1 (SEQ ID NO: 1 or 2), PARP2 (SEQ ID NO: 3), PARP3 (SEQ ID NO: 4), PARP3.2 (SEQ ID NO: 5), PARP3.3 (SEQ ID NO: 6), PARP4 (SEQ ID NO: 7), PARP5A (SEQ ID NO: 8 or 9), PARP5B (SEQ ID NO: 10), PARP6 (SEQ ID NO: 11), PARP7 (SEQ ID NO: 12), PARP8 (SEQ ID NO: 13), PARP9 (SEQ ID NO: 14), PARP10 (SEQ ID NO: 15), PARP10.2 (SEQ ID NO: 16), PARP11 (SEQ ID NO: 17), PARP12 (SEQ ID NO: 18), PARP13.1 (SEQ ID NO: 19), PARP13.2 (SEQ ID NO: 20), PARP14 (SEQ ID NO: 21), PARP15.1 (SEQ ID NO: 22), PARP15.2 (SEQ ID NO: 23), and PARP16 (SEQ ID NO: 24). A PARP nucleic acid encodes a protein that has a catalytic activity of attaching an ADP-ribose to a substrate (e.g., protein, DNA, RNA, lipid, or small molecule) or attaching one or more ADP-ribose molecules to an ADP-ribose molecule already attached to the substrate (e.g., protein, DNA, RNA, lipid, or small molecule) to create poly-ADP ribose. A PARP nucleic acid may encode a protein having additional activities to those described above (e.g., mediates increased stress granule formation, role in progression through mitosis or cytokinesis, and modulation (e.g., increase or decrease) of RNAi function).

By “poly-ADP ribose polymerase protein” or “PARP protein” is meant polypeptide containing a sequence having at least 80% identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100% identity) to a protein encoded by a nucleic acid sequence containing the sequence of PARP1 (SEQ ID NO: 1 or 2), PARP2 (SEQ ID NO: 3), PARP3 (SEQ ID NO: 4), PARP3.2 (SEQ ID NO: 5), PARP3.3 (SEQ ID NO: 6), PARP4 (SEQ ID NO: 7), PARP5A (SEQ ID NO: 8 or 9), PARP5B (SEQ ID NO: 10), PARP6 (SEQ ID NO: 11), PARP7 (SEQ ID NO: 12), PARP8 (SEQ ID NO: 13), PARP9 (SEQ ID NO: 14), PARP10 (SEQ ID NO: 15), PARP10.2 (SEQ ID NO: 16), PARP11 (SEQ ID NO: 17), PARP12 (SEQ ID NO: 18), PARP13.1 (SEQ ID NO: 19), PARP13.2 (SEQ ID NO: 20), PARP14 (SEQ ID NO: 21), PARP15.1 (SEQ ID NO: 22), PARP15.2 (SEQ ID NO: 23), and PARP16 (SEQ ID NO: 24). A PARP protein may contain one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) post-translational modifications, e.g., phosphorylation and ADP-ribosylation (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 ADP-ribose molecules) on one or more amino acid residues. Post-translation modification of a PARP protein may occur within a cell (e.g., a transgenic cell

described above) or in vitro using purified enzymes. PARP protein activity assays may be performed as described herein.

By “poly-ADP ribose polymerase fusion protein” or “PARP fusion protein” is meant a polypeptide containing a polypeptide tag and a sequence having at least 80% identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100% identity) to a protein encoded by one or more of PARP1 (SEQ ID NO: 1 or 2), PARP2 (SEQ ID NO: 3), PARP3 (SEQ ID NO: 4), PARP3.2 (SEQ ID NO: 5), PARP3.3 (SEQ ID NO: 6), PARP4 (SEQ ID NO: 7), PARP5A (SEQ ID NO: 8 or 9), PARP5B (SEQ ID NO: 10), PARP6 (SEQ ID NO: 11), PARP7 (SEQ ID NO: 12), PARP8 (SEQ ID NO: 13), PARP9 (SEQ ID NO: 14), PARP10 (SEQ ID NO: 15), PARP10.2 (SEQ ID NO: 16), PARP11 (SEQ ID NO: 17), PARP12 (SEQ ID NO: 18), PARP13.1 (SEQ ID NO: 19), PARP13.2 (SEQ ID NO: 20), PARP14 (SEQ ID NO: 21), PARP15.1 (SEQ ID NO: 22), PARP15.2 (SEQ ID NO: 23), and PARP16 (SEQ ID NO: 24). The polypeptide tag of a PARP fusion protein may be located at the N- and/or C-terminus of the protein. The polypeptide tag may contain one or more of a fluorescent protein (e.g., a green fluorescence protein), a peptide epitope recognized by specific antibodies, a protein that is bound by a partner binding protein with high affinity (e.g., biotin and streptavidin), a His₆-tag, or one or more (e.g., 1, 2, 3, 4, 5, 6, or 7) protease recognition sequence(s) (e.g., one or more of a TEV protease or Factor Xa protease recognition sequence). The PARP fusion proteins of the invention may be purified using antibodies specific for the polypeptide tag. For example, antibodies specific for the polypeptide tag or proteins that bind specifically to the protein sequence in the polypeptide tag may be bound to a bead (e.g., a magnetic bead) or polymer surface in order to allow for the purification of the PARP fusion protein. A PARP fusion protein may also be purified and subsequently treated with one or more (e.g., 1, 2, or 3) protease(s) to remove the polypeptide tag from the PARP fusion protein. A PARP fusion protein preferably has the same cellular localization and biological activity as the wild-type PARP protein. Methods for the generation and purification of PARP fusion proteins are described herein.

By “PARP activator” or “poly-ADP-ribose polymerase activator” is meant an agent that increases the expression (e.g., mRNA or protein level) and/or one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) biological activities of one or more PARPs. For example, a PARP activator may increase the level of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) PARP nucleic acids or PARP proteins (described above). A PARP activator may increase one or more biological activities of a PARP protein including, but not limited to, the ability to attach a poly-ADP-ribose molecule to one or more substrate(s) (e.g., a protein, DNA molecule, RNA molecule, lipid, or small molecule), the ability to promote formation of a stress granule, the ability to nucleate the formation of a stress granule, the ability to disassemble a stress granule, the ability to decrease stress granule assembly, the ability to localize to a stress granule, the ability of a PARP protein to bind to one or more of its substrates, the ability of a PARP protein to localize to the nucleus or the mitotic spindle, the ability to promote cell proliferation, and the ability to promote progression through cytokinesis. Specific PARP activators include nucleic acids encoding one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) PARPs or the catalytic domains of one or more PARPs. For example, a PARP activator may be a nucleic acid containing a nucleic acid sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARP1 (SEQ ID NO: 1 or 2), PARP2 (SEQ ID NO: 3), PARP3 (SEQ ID NO: 4), PARP3.2

(SEQ ID NO: 5), PARP3.3 (SEQ ID NO: 6), PARP4 (SEQ ID NO: 7), PARP5A (SEQ ID NO: 8 or 9), PARP5B (SEQ ID NO: 10), PARP6 (SEQ ID NO: 11), PARP7 (SEQ ID NO: 12), PARP8 (SEQ ID NO: 13), PARP9 (SEQ ID NO: 14), PARP10 (SEQ ID NO: 15), PARP10.2 (SEQ ID NO: 16), PARP11 (SEQ ID NO: 17), PARP12 (SEQ ID NO: 18), PARP13.1 (SEQ ID NO: 19), PARP13.2 (SEQ ID NO: 20), PARP14 (SEQ ID NO: 21), PARP15.1 (SEQ ID NO: 22), PARP15.2 (SEQ ID NO: 23), and PARP16 (SEQ ID NO: 24). Specific PARP activators may increase the expression and/or one or more (e.g., 1, 2, 3, 4, or 5) biological activities of a specific PARP or a specific subset of PARPs (e.g., one or more of PARP5A, PARP12, PARP13.1, PARP13.2, and PARP15; PARP11; and one or more of PARP1, PARP2, PARP5A, PARP5B, PARP7, PARP8, PARP14, and PARP16).

By “PARP inhibitor” or “poly-ADP-ribose polymerase inhibitor” is meant an agent that decreases the expression (e.g., mRNA or protein level) and/or one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) biological activities of one or more PARPs. For example, a PARP inhibitor may decrease the level of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) PARP nucleic acids or PARP proteins (described above). A PARP inhibitor may decrease one or more (e.g., 1, 2, 3, 4, or 5) biological activities of a PARP protein including, but not limited to, the ability to attach a poly-ADP-ribose molecule to a substrate (e.g., a protein, DNA molecule, RNA molecule, lipid, or small molecule), the ability to promote formation of a stress granule, the ability to nucleate the formation of a stress granule, the ability to disassemble a stress granule, the ability to decrease stress granule assembly, the ability to localize to a stress granule, the ability of a PARP protein to bind to one or more of its substrates, the ability of a PARP protein to localize to the nucleus or the mitotic spindle, the ability to promote cell proliferation, and the ability to promote progression through cytokinesis. Specific PARP inhibitors include antibody or antibody fragments that specifically bind one or more PARP proteins (described herein), one or more RNA aptamers (e.g., RNAi molecules; e.g., SEQ ID NOS: 40 and 43-136), and one or more small molecules. Specific PARP inhibitors may decrease the expression and/or one or more biological activities (e.g., 1, 2, 3, 4, or 5) of a specific PARP or a specific subset of PARPs (e.g., one or more (e.g., 1, 2, 3, 4, or 5) of PARP5A, PARP12, PARP13.1, PARP13.2, and PARP15; PARP11; and one or more (e.g., 1, 2, 3, 4, 5, 6, 7, or 8) of PARP1, PARP2, PARP5A, PARP5B, PARP7, PARP8, PARP14, and PARP16).

By “Poly-ADP-ribose polymerase 11 activator” is meant an agent that increases the expression (e.g., mRNA or protein level) and/or one or more (e.g., 1, 2, 3, 4, or 5) biological activities of PARP11. For example, a PARP11 activator may increase the level of one or more (e.g., 1, 2, 3, 4, or 5) nucleic acids containing a sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARP11 (SEQ ID NO: 17), and/or increase the level of one or more (e.g., 1, 2, 3, 4, or 5) polypeptides encoded by these nucleic acids. A PARP11 activator may increase one or more (e.g., 1, 2, 3, 4, or 5) biological activities of a PARP11 protein including, but not limited to, the ability to attach a poly-ADP-ribose molecule to a substrate (e.g., a protein, DNA molecule, RNA molecule, lipid, or small molecule), the ability to prevent or reduce the rate of formation of a stress granule, the ability to prevent the nucleation of a stress granule, the ability to disassemble a stress granule, the ability to decrease stress granule assembly, the ability to localize to a stress granule, or the ability of PARP11 protein to bind to one or more of its substrates. Specific PARP11 activators

include nucleic acids encoding PARP11 or the catalytic domain of PARP11. For example, a PARP activator may be a nucleic acid containing a nucleic acid sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARP11 (SEQ ID NO: 17). Specific PARP11 activators may increase the expression and/or one or more biological activities of a PARP11, while having little or no effect on the expression and/or one or more biological activities of other PARP proteins.

By “poly-ADP-ribose polymerase-11 inhibitor” or “PARP11 inhibitor” is meant an agent that decreases the expression (e.g., mRNA or protein level) and/or one or more (e.g., 1, 2, 3, 4, or 5) biological activities of PARP11. For example, a PARP11 inhibitor may decrease the level of one or more (e.g., 1, 2, 3, 4, or 5) nucleic acids containing a sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARP11 (SEQ ID NO: 17), or decrease the level of one or more (e.g., 1, 2, 3, 4, or 5) polypeptides encoded by these nucleic acids. A PARP11 inhibitor may decrease one or more (e.g., 1, 2, 3, 4, or 5) biological activities of a PARP11 protein including, but not limited to, the ability to attach a poly-ADP-ribose molecule to a substrate (e.g., a protein, DNA molecule, RNA molecule, lipid, or small molecule), the ability to prevent or reduce the rate of formation of a stress granule, the ability to prevent the nucleation of a stress granule, the ability to disassemble a stress granule, the ability to decrease stress granule assembly, the ability to localize to a stress granule, or the ability of PARP11 protein to bind to one or more of its substrates. Specific PARP11 inhibitors include antibody or antibody fragments that specifically bind to PARP11 protein, one or more RNA aptamers (e.g., RNAi molecules; e.g., SEQ ID NOS: 91-98), and one or more small molecules. Specific PARP11 inhibitors decrease the expression and/or one or more (e.g., 1, 2, 3, 4, or 5) biological activities of PARP11, while having little or no effect on the expression and/or activity of other PARP proteins.

By “PARP biological activity” is meant one or more (e.g., 1, 2, 3, 4, or 5) of the ability of a PARP protein or PARP fusion protein to catalyze the attachment of a single ADP-ribose to a target substrate (e.g., a protein, DNA, RNA, lipid, or small molecule), the ability to attach one or more ADP-ribose molecules to a ADP-ribose molecule already attached to a substrate, the ability to add a branched ADP-ribose molecule to a pre-existing poly-ADP-ribose, the ability to localize to the cell nucleus, the ability to localize to stress granules, the ability to catalyze the formation or nucleate stress granules, the ability to catalyze the disassembly of stress granules, the ability to promote cell division or progression through mitosis, or the ability to activate or inhibit RNAi activity in the cell. Specific PARP proteins have a different subset of biological activities. For example, PARP1, PARP2, PARP5A, PARP5B, PARP7, PARP8, PARP14, and PARP16 have the ability to localize to the nucleus and play a role in mitosis and cell division. PARP 5A, PARP12, PARP13.1, PARP13.2, and PARP-15 have the ability to localize to stress granules and play a role in the formation or nucleation of stress granules. PARP11 has the ability to localize to stress granules and plays a role in inhibiting stress granule formation or increasing the disassembly of stress granules. PARP13 inhibits the activity of RNAi in the cell. An additional PARP activity is “auto-modification” or “auto-modulation,” that is, attachment of one or more ADP-ribose molecules to itself. Such auto-modulation of a PARP protein may result in an increase or decrease

in any of the above-listed PARP activities. Assays for the measurement of the activity of each specific PARP are described herein.

By “polypeptide tag” is meant a protein sequence that is located at the 5' and/or 3' end of a polypeptide sequence of an expressed protein (e.g., one or more PARP proteins as described herein). A polypeptide tag may include one or more of a protease recognition sequence (e.g., 1, 2, 3, 4, 5, or 6 of the same or different protease recognition sequences), a epitope tag (e.g., 1, 2, 3, 4, or 5 epitope tags), a peptide that has a high affinity binding partner (e.g., biotin and streptavidin), or one or more (e.g., 1, 2, 3, or 4) tag(s) which aids in protein purification (e.g., a His₆ tag). The polypeptide tag may later be cleaved from the purified fusion protein by incubation with one or more (e.g., 1, 2, 3, or 4) protease(s) which cleaves the fusion protein at one or more protease recognition sequence(s) (e.g., 1, 2, 3, 4, 5, 6, or 7) within the sequence of the polypeptide tag. Examples of polypeptide tags are described herein.

By “positioned 3'” is meant a second nucleic acid sequence that is located after the 3' terminus of a first nucleic acid sequence (the second nucleotide sequence starts at the nucleotide following the 3' terminus of the first sequence) or the second nucleic acid sequence begins at a nucleotide that follows the 3' terminus of the first nucleic acid (e.g., the second nucleotide sequence starts at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, or 400 nucleotides following the 3' terminus of the first nucleic acid).

By “positioned 5'” is meant a second nucleic acid sequence that is located before the 5' terminus of a first nucleic acid sequence (the second nucleotide sequence ends at the nucleotide preceding the 5' terminus of the first sequence) or the second nucleic acid sequence ends at a nucleotide that precedes the 5' terminus of the first nucleic acid (e.g., the second nucleotide sequence ends at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, or 400 nucleotides before the 5' terminus of the first nucleic acid).

By the term “propensity to develop disease” is meant the calculated probability of a subject (e.g., a human) to develop a disease (e.g., a stress granule-related disorder or cancer). The probability of developing a disease may be calculated based on a number of factors including a variety of health indicators (e.g., blood pressure, cholesterol, and levels of pro-inflammatory cytokines), biological factors (e.g., weight, age, and sex), and genetic susceptibility to disease (e.g., expression of a heritable mutation in a gene, expression of a polymorphic sequence associated with a disease, and expression of an allele associated with a disease). The propensity to develop disease in a specific patient population may be compared to a different patient population (e.g., a patient population not receiving a therapy).

By the term “protease recognition sequence” is meant a short peptide sequence that is recognized as a substrate and cleaved by one or more (e.g., 1, 2, 3, 4, or 5) proteases. Protease target sequences are often 3-20 amino acids in length and often require certain amino acids to be located at specific positions within the target sequence, while any amino acid may be placed at other positions within the target sequence. For example, the protease recognition sequence for TEV protease is Glu-X-X-Tyr-X-Gln-Ser (SEQ ID NO: 26), where X represents a position that may be filled by any amino acid. Additional examples of protease recognition sequences are known in the art and include, without limitation, factor Xa (Ile-Glu/Asp-Gly-Arg), Ala-64 subtilisin (Gly-Ala-His-Arg), clostripain (Arg and Lys-Arg), collagenase (Pro-Val-Gly-Pro), enterokinase (Asp-Asp-Asp-Asp-Lys), renin (Pro-

Phe-His-Leu-Leu), and α -thrombin (Leu-Val-Pro-Arg-Gly-Ser). One or more of the same or different protease recognition sequence(s) may be included in the polypeptide tag of any of the PARP, PARG, or ARH3 fusion proteins described herein. A protease recognition sequence may be placed 5' or 3' to an amino acid sequence to be removed from the protein. The polypeptide sequence of the protease recognition sequence may directly abut the sequence encoding a PARP or may be separated from the remaining coding sequence by one or more amino acids (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 40, or 50 amino acids). An amino acid sequence that may be removed from the protein may include one or more antigenic sequence(s), a His₆-tag, a fluorescent protein, a peptide sequence that has high affinity to a second protein that was used to purify the protein (e.g., His₆ tag or hemagglutinin tag), or a peptide sequence that was used to stabilize the protein during purification (e.g., albumin).

By the term "purified" is meant purified from other common components normally present within the cell. For example, a purified protein is purified away from the other cellular proteins, nucleic acids, and small metabolites present within the cell. A purified protein is at least 85% pure by weight (e.g., at least 90%, 95%, 96%, 97%, 98%, 99%, or even 100% pure) from other proteins, nucleic acids, or small metabolites present in the cell. A purified nucleic acid is at least 85% free of other contaminating nucleic acid molecules or adjoining sequences found in the cell.

By the term "rate of proliferation" is meant the rate of a cell or a cell population to undergo successive cell divisions. The rate of cell proliferation of a cell or cell population may be measured by methods known in the art including cell counting (e.g., by microscopic techniques) and the incorporation of labeled nucleotides into newly synthesized DNA (e.g., incorporation of ³H-thymidine). The rate of proliferation of a cell or cell population treated with one or more agent(s) (e.g., one or more PARP activators or PARP inhibitors) may be compared to a control cell or cell population not treated with the agent.

By the term "reduce the likelihood of developing" is meant a reduction (e.g., at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 50%, 60%, 70%, 80%, 90%, or 95%) for an individual or a patient population in the chance or rate of developing a specific disease by administering one or more therapeutic agent(s) compared to an individual or patient population not receiving the therapeutic agent. The methods of the invention may also reduce the likelihood of developing one or more (e.g., 1, 2, 3, 4, or 5) symptoms of a stress granule-related disorder or reduce the likelihood of developing one or more (e.g., 1, 2, 3, 4, or 5) symptoms of cancer in a patient population or an individual receiving one or more therapeutic agent(s).

By the term "RNAi" is meant a short double-stranded RNA molecule that mediates the down-regulation of a target mRNA in a cell. An RNAi molecule is typically 15 to 32 nucleotides in length. RNAi molecules are also known as siRNAs, small RNAs, or microRNAs. The design and therapeutic effectiveness of RNAi molecules is described in McCaffrey et al. (*Nature* 418:38-39, 2002). The RNAi molecules are at least 15 nucleotides, preferably, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35 nucleotides in length and even up to 50 or 100 nucleotides in length (inclusive of all integers in between). Non-limiting examples of RNAi molecules are at least 80% identical (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100% identical) to or complementary to the translational start sequence or the nucleic acid sequence encoding the first 10,

20, 30, 40, 50, 60, 70, 80, 90, or 100 amino acids of a PARP, PARG, or ARH3 selected from a nucleic acid sequence containing a sequence at least 80% identical to one of PARP1 (SEQ ID NO: 1 or 2), PARP2 (SEQ ID NO: 3), PARP3 (SEQ ID NO: 4), PARP3.2 (SEQ ID NO: 5), PARP3.3 (SEQ ID NO: 6), PARP4 (SEQ ID NO: 7), PARP5A (SEQ ID NO: 8 or 9), PARP5B (SEQ ID NO: 10), PARP6 (SEQ ID NO: 11), PARP7 (SEQ ID NO: 12), PARP8 (SEQ ID NO: 13), PARP9 (SEQ ID NO: 14), PARP10 (SEQ ID NO: 15), PARP10.2 (SEQ ID NO: 16), PARP11 (SEQ ID NO: 17), PARP12 (SEQ ID NO: 18), PARP13.1 (SEQ ID NO: 19), PARP13.2 (SEQ ID NO: 20), PARP14 (SEQ ID NO: 21), PARP15.1 (SEQ ID NO: 22), PARP15.2 (SEQ ID NO: 23), PARP16 (SEQ ID NO: 24), PARG (SEQ ID NO: 42), or ARH3 (SEQ ID NO: 41). An RNAi molecule may target any part of the sequence encoding the target protein (e.g., any part of an mRNA encoding one of the above listed PARP, PARG, or ARH3 proteins).

The specific requirements and modifications of small RNA are known in the art and are described, for example in PCT Publication No. WO01/75164, and U.S. Application Publication Nos. 20060134787, 20050153918, 20050058982, 20050037988, and 20040203145, the relevant portions of which are herein incorporated by reference. siRNAs can also be synthesized or generated by processing longer double-stranded RNAs, for example, in the presence of the enzyme dicer under conditions in which the dsRNA is processed to RNA molecules of about 17 to about 26 nucleotides. siRNAs can also be generated by expression of the corresponding DNA fragment (e.g., a hairpin DNA construct). Generally, the siRNA has a characteristic 2- to 3-nucleotide 3' overhanging ends, preferably these are (2'-deoxy) thymidine or uracil. The siRNAs typically comprise a 3' hydroxyl group. Single-stranded siRNAs or blunt-ended dsRNA may also be used. In order to further enhance the stability of the RNA, the 3' overhangs may be stabilized against degradation. For example, the RNA may be stabilized by including purine nucleotides, such as adenosine or guanosine. Alternatively, substitution of pyrimidine nucleotides by modified analogs, e.g., substitution of uridine 2-nucleotide overhangs by (2'-deoxy)thymidine is tolerated and does not affect the efficiency of RNAi. The absence of a 2' hydroxyl group significantly enhances the nuclease resistance of the overhang in tissue culture medium.

siRNA molecules can also be obtained through a variety of protocols including chemical synthesis or recombinant production using a *Drosophila* in vitro system. They can be commercially obtained from companies such as Dharmacon Research Inc. or Xeragon Inc., or they can be synthesized using commercially available kits such as the Silencer™ siRNA Construction Kit from Ambion (catalog number 1620) or HiScribe™ RNAi Transcription Kit from New England BioLabs (catalog number E2000S).

Alternatively siRNA can be prepared using standard procedures for in vitro transcription of RNA and dsRNA annealing procedures such as those described in Elbashir et al. (*Genes & Dev.*, 15:188-200, 2001), Girard et al. (*Nature* 442:199-202, 2006), Aravin et al. (*Nature* 442:203-207, 2006), Grivna et al. (*Genes Dev.* 20:1709-1714, 2006), and Lau et al. (*Science* 313:305-306, 2006). siRNAs may also be obtained by incubation of dsRNA that corresponds to a sequence of the target gene in a cell-free *Drosophila* lysate from syncytial blastoderm *Drosophila* embryos under conditions in which the dsRNA is processed to generate siRNAs of about 21 to about 23 nucleotides, which are then isolated using techniques known to those of skill in the art. For example, gel electrophoresis can be used to separate the 21-23 nt RNAs and the RNAs can then be eluted from the gel

slices. In addition, chromatography (e.g., size exclusion chromatography), glycerol gradient centrifugation, and affinity purification with antibody can be used to isolate the small RNAs.

Short hairpin RNAs (shRNAs), as described in Yu et al. (*Proc. Natl. Acad. Sci. U.S.A.* 99:6047-6052, 2002) or Pad-dison et al. (*Genes & Dev.* 16:948-958, 2002), incorporated herein by reference, may also be used. shRNAs are designed such that both the sense and antisense strands are included within a single RNA molecule and connected by a loop of nucleotides (3 or more). shRNAs can be synthesized and purified using standard in vitro T7 transcription synthesis as described above and in Yu et al. (*supra*). shRNAs can also be subcloned into an expression vector that has the mouse U6 promoter sequences which can then be transfected into cells and used for in vivo expression of the shRNA.

A variety of methods and reagents are available for transfection, or introduction, of dsRNA into mammalian cells including but not limited to: TransIT-TKO™ (Mirus, Cat. # MIR 2150), Transmessenger™ (Qiagen, Cat. #301525), Oli-gofectamine™ and Lipofectamine™ (Invitrogen, Cat. #MIR 12252-011 and Cat. #13778-075), siPORT™ (Ambion, Cat. #1631), and DharmaFECT™ (Fisher Scientific, Cat. #T-2001-01). Agents are also commercially available for electroporation-based methods for transfection of siRNA, such as siPORTer™ (Ambion Inc. Cat. #1629). Microinjection techniques can also be used. The small RNA can also be transcribed from an expression construct introduced into the cells, where the expression construct includes a coding sequence for transcribing the small RNA operably-linked to one or more transcriptional regulatory sequences. Where desired, plasmids, vectors, or viral vectors can also be used for the delivery of dsRNA or siRNA and such vectors are known in the art. Protocols for each transfection reagent are available from the manufacturer. Additional methods are known in the art and are described, for example in U.S. Patent Application Publication No. 20060058255.

By the term “specifically binds” is meant a protein, nucleic acid (e.g., DNA or RNA), or molecule that binds one or more target molecules (e.g., polypeptides, DNA molecules, or RNA molecules) present in a cell, while not binding the majority of other proteins, DNA molecules, RNA molecules, or small molecules present within a cell, cell lysate, extracellular medium, or biological sample. For example, an antibody provided by the invention may bind to a single PARP, PARG, or ARH protein, a PARP, PARG, or ARH3 fusion protein, or may bind more than one (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 or more) PARP, PARG, or ARH3 proteins and/or PARP, PARG, or ARH3 fusion proteins in a cell, cell lysate, extracellular medium, or biological sample.

By “substrate” or “solid surface” is meant a surface on which a moiety or protein is covalently attached which allows for the binding and/or purification of a PARP, PARG, and/or ARH3 fusion protein. The PARP, PARG, and/or ARH3 fusion protein will bind to the substrate or solid surface through its polypeptide tag. Moieties or peptides covalently attached to the substrate or solid surface include, but are not limited to, monoclonal or polyclonal antibodies specific for an antigenic peptide in the polypeptide tag (e.g., anti-GFP antibody binding to GFP in the polypeptide tag), specific metal complexes bound by a peptide located in the polypeptide tag (e.g., Ni²⁺ binding to a His₆ polypeptide tag), or a specific binding protein for a peptide located in the polypeptide tag (e.g., IgG binding to a ZZ-domain in the polypeptide tag). Examples of a substrate or solid surface include, but are not limited to, a bead (e.g., a magnetic bead), a surface in a multi-well plate,

and beads in column (e.g., column chromatography). One or more PARP, PARG, and/or ARH3 protein(s) and/or PARP, PARG, and/or ARH3 fusion protein(s) may be bound to a substrate or solid surface and eluted from the substrate or solid surface by contacting the substrate or solid surface with an elution buffer (e.g., a high salt elution buffer), a ligand that competes for binding to the substrate or solid surface, or competes for binding to the polypeptide tag (e.g., a non-bound antibody that specifically binds to the protein in the polypeptide tag), or by treating the bound fusion protein with a protease that recognizes the one or more (e.g., 1, 2, 3, 4, 5, 6, or 7) specific cleavage recognition sequence(s) found in the polypeptide tag.

By the term “stress granule-related disorder” is meant any disorder that is characterized or in part caused by the activity or formation of stress granules in a specific type of cell or cell population. Non-limiting examples of stress granule-related disorders include cardiovascular disorders (e.g., an aneurysm, angina, atherosclerosis, stroke, cerebrovascular disease, congestive heart failure, coronary artery disease, myocardial disease, peripheral vascular disease, granulomatous myocarditis, chronic myocarditis, myocardial infarction, and primary hypertrophic cardiomyopathy), inflammatory disorders (e.g., autoimmune diseases, asthma, allergic intraocular inflammatory diseases, arthritis, atopic dermatitis, atopic eczema, cirrhosis, Crohn’s disease, ulcerative colitis, diabetes, hemolytic anemia, inflammatory dermatosis, an inflammatory bowel disorder, systemic lupus erythematosus, psoriasis, and rheumatoid arthritis, Wegener’s granulomatosis, Hashimoto’s thyroiditis, chronic pancreatitis, and reactive lymphoid hyperplasia), neurological disorders (e.g., multiple sclerosis, Alzheimer’s disease, Parkinson’s disease, Huntington’s disease, amyotrophic lateral sclerosis, retinosa pigmentosa, macular degeneration, traumatic brain injury, stroke, and peripheral neuropathy), and ischemia-reperfusion injury (e.g., stroke). A stress granule-related disorder is typically characterized by a disease etiology that involves oxidative stress or the production of oxygen-based radicals in a tissue over the progression of the disease. Methods for the diagnosis of several stress granule-related disorders are known in the art.

By the term “symptoms of a stress-granule related disorder” is meant one or more (e.g., 1, 2, 3, 4, or 5) of the physical manifestations of a stress-granule related disorder. Non-limiting examples of symptoms of a stress-granule related disorder include pain, swelling, inflammation, loss of cognition, loss of vision, loss of coordination, difficulty breathing, airway constriction, artery occlusion, diarrhea, elevated blood glucose, increased levels of pro-inflammatory cytokines, increased protein aggregates or deposits, and increased cell death (e.g., apoptosis or necrosis).

By the term “symptoms of cancer” is meant one or more (e.g., 1, 2, 3, 4, or 5) of the physical manifestations of cancer. Non-limiting examples of symptoms of cancer include blood in urine, pain or burning upon urination, cloudy urine, pain in bone, fractures in bones, fatigue, weight loss, repeated infections, nausea, vomiting, constipation, numbness in the legs, bruising, dizziness, drowsiness, abnormal eye movements, changes in vision, changes in speech, headaches, thickening of a tissue, rectal bleeding, abdominal cramps, loss of appetite, fever, enlarged lymphnodes, persistent cough, blood in sputum, lung congestion, itchy skin, lumps in skin, abdominal swelling, vaginal bleeding, jaundice, heartburn, indigestion, cell proliferation, and loss of regulation of controlled cell death.

By the term “target protein” or “substrate protein” is meant a protein that is bound by one or more (e.g., 1, 2, 3, 4, or 5)

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PARP protein(s), PARP protein(s), ARH3 protein(s), PARP fusion protein(s), PARP fusion protein(s), and/or ARH3 fusion protein(s); covalently modified by attachment of a ADP-ribose molecule by the activity of one or more (e.g., 1, 2, 3, 4, or 5) PARP protein(s) or PARP fusion protein(s); or contains a poly-ADP-ribosyl group that is hydrolyzed by the activity of one or more (e.g., 1, 2, 3, 4, or 5) PARP proteins, PARP fusion proteins, ARH3 proteins, or ARH3 fusion proteins. A target or substrate protein may be co-localized in the nucleus or in a stress granule, and/or may localize to the mitotic spindle during cytokinesis. A target protein or substrate protein may localize to different structures or organelles within a cell during different stages of the cell cycle (e.g., interphase, S-phase, prophase, metaphase, telophase, and anaphase) and may have an activity in the formation, nucleation, or disassembly of stress granules, an activity in cell proliferation or progression through cytokinesis, or an activity in the regulation of miRNA or RNAi activity. A target or substrate protein may be a PARP, PARP, or ARH3 protein (described herein).

By the term "transgenic cell" is meant a cell expressing one or more nucleic acids introduced by recombinant DNA technology. For example, a transgenic cell may express a nucleic acid encoding one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or 18) of the presently described PARP, PARP, and/or ARH3 proteins and/or one or more of the PARP, PARP, and/or ARH3 fusion proteins. A transgenic cell may be a mammalian cell (e.g., a mouse, rat, monkey, or human cell), a bacterial cell, a fungal cell, a yeast cell, or a plant cell. The transgenic cell may express the introduced nucleic acids from an inducible promoter or a constitutive promoter. The transgenic cell may also be located within a transgenic animal or may be cultured in tissue culture. The introduced one or more nucleic acid(s) may be integrated in the chromosome of a cell or may be expressed from a plasmid.

By "ZZ-domain" is meant a polypeptide sequence encoded by a nucleic acid having at least 80% identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100% identity) to the *Staphylococcus aureus* protein A domain encoded by SEQ ID NO: 27. The ZZ domain has the ability to bind to Fcγ (the constant region of IgG involved in effector functions) and Fab (the Ig fragment responsible for antigen recognition). The specific structure and binding properties of the ZZ-domain are described in Graille et al. (*Proc. Natl. Acad. Sci. U.S.A.* 97:5399-5404, 2000) and Roben et al. (*J. Immunol.* 154:6437-6445, 1995). Expression of the ZZ-domain in the polypeptide tag allows for the purification of a fusion protein (e.g., one or more PARP fusion proteins as described herein) by the use of an Fc-containing protein (e.g., IgG).

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

The application file contains drawings executed in color (FIGS. 2, 5, 9-11, 16-18, and 20-22). Copies of this patent or patent application with color drawings will be provided by the Office upon request and payment of the necessary fee.

FIG. 1 is a picture of the chemical structure of nicotinamide adenine dinucleotide (NAD⁺) and poly-ADP ribose.

FIG. 2 is a set of micrographs showing the mitotic localization of poly-ADP ribose in HeLa cells during G2-M, prophase, prometaphase (P-M), metaphase, anaphase, and cytokinesis stages of the cell cycle using fluorescence micros-

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copy following staining with rabbit anti-PAR antibodies labeled with Alexa 488 and X-rhodamine NHS esters.

FIG. 3 is a set of schematic diagrams showing the domain organization of PARP1, PARP2, PARP3, PARP4, tankyrase 1 (PARP5A), tankyrase 2 (PARP5B), TipARP (PARP7), PARP12, PARP13, PARP9, PARP14, PARP15, PARP10, PARP11, PARP6, PARP8, and PARP16.

FIG. 4 is a diagram of the pEGFP-C1 vector (SEQ ID NO: 28) (Invitrogen) showing the CMV promoter, the EGFP sequence, the multiple cloning site (MCS), and the SV40 poly A sequence. Also shown is the polylinker sequence (SEQ ID NO: 29).

FIG. 5 is an immunoblot showing the expression and relative size of the PARP-GFP fusion proteins of PARP1, PARP2, PARP3, PARP4, PARP5A, PARP5B, PARP6, PARP7, PARP8, PARP9, PARP10, PARP11, PARP12, PARP13, PARP14, PARP15, and PARP16 expressed in HeLa Kyoto cells transfected with pEGFP-C1 plasmids containing a nucleic acid encoding each respective PARP-GFP fusion protein. The immunoblot was developed using a rabbit anti-GFP polyclonal antibody.

FIG. 6 is a set of micrographs showing the localization of different PARP-GFP fusion proteins in asynchronous HeLa Kyoto cells transfected with a pEGFP-C1 plasmid containing a nucleic acid encoding a PARP-GFP protein. The transfected cells were immunostained with rabbit anti-GFP polyclonal antibody and fluorescently-labeled secondary Alexa Fluor 594 or 488 antibody (Invitrogen), and visualized using fluorescence microscopy. The localization of PARP1-GFP, PARP2-GFP, PARP3-GFP, PARP7-GFP, PARP12-GFP, PARP13-GFP, PARP9-GFP, PARP14-GFP, PARP15-GFP, PARP6-GFP, PARP8-GFP, PARP16-GFP, PARP4-GFP, PARP10-GFP, PARP11-GFP, PARP5A-GFP, and PARP5B-GFP fusion proteins is shown.

FIG. 7 is two sets of micrographs showing the localization of different PARP-GFP fusion proteins in asynchronous hTERT-RPE and HeLa Kyoto cells transfected with a pEGFP-C1 plasmid containing a nucleic acid encoding a PARP-GFP protein. The transfected cells were immunostained with rabbit anti-GFP polyclonal antibody and fluorescently-labeled Alexa Fluor 594 or 488 antibodies (Invitrogen), and visualized using fluorescence microscopy. The localization of PARP1-GFP, PARP2-GFP, PARP3-GFP, PARP4-GFP, PARP5A-GFP, PARP5B-GFP, PARP6-GFP, PARP7-GFP, PARP8-GFP, PARP9-GFP, PARP10-GFP, PARP11-GFP, PARP12-GFP, PARP13-GFP, PARP14-GFP, PARP15-GFP, and PARP16-GFP fusion proteins is shown for each cell type.

FIG. 8 is a set of micrographs from asynchronous HeLa Kyoto cells transfected with a pEGFP-C1 plasmid containing a nucleic acid encoding a PARP-GFP protein following immunostaining with primary rabbit antibodies raised against each specific PARP and fluorescently-labeled Alexa Fluor 594 or 488 antibodies (Invitrogen). The localization of PARP1, PARP2, PARP3, PARP4, PARP5A, PARP5B, PARP6, PARP7, PARP8, PARP9, PARP10, PARP11, PARP12, PARP13.1, PARP13.2, PARP14, PARP15, and PARP16 is shown.

FIG. 9 is a set of micrographs showing the localization of each PARP-GFP fusion protein during S-phase and mitosis in transfected HeLa Kyoto cells. In each experiment, HeLa Kyoto cells were transfected using Lipofectamine 2000 with a specific PARP-GFP expression vector (pEGFP-C1) and were arrested in mitosis or S-phase by treatment with 100 nM nocodazole or 5 μg/mL aphidicolin for 12 hours. The resulting treated cells were immunostained with rabbit anti-GFP polyclonal antibodies and fluorescently-labeled Alexa Fluor

594 or 488 antibodies (Invitrogen), and visualized using fluorescence microscopy. S-phase-arrested cells were also stained with EdU and DAPI, and mitosis-arrested cells were further stained with tubulin and DAPI.

FIG. 10 is a set of micrographs showing the localization of overexpressed PARP16-GFP in the endoplasmic reticulum of HeLa Kyoto cells transfected with a pEGFP-C1 plasmid encoding a PARP16-GFP fusion protein (middle panel) and the phenotype of HeLa Kyoto cells transfected with an RNAi targeting endogenous PARP16 (right panel). The left panel shows untransfected HeLa Kyoto cells stained with anti-calnexin antibodies, secondary fluorescently-labeled antibodies (Alexa Fluor 594 or 488 antibodies (Invitrogen)), and DAPI. The middle panel the localization of PARP16-GFP and calnexin in HeLa Kyoto cells transfected with a pEGFP-C1 plasmid expressing a PARP16-GFP fusion protein following staining with anti-calnexin, anti-GFP, Alexa Fluor 594 or 488 antibodies (Invitrogen), and DAPI. The right panel shows the phenotype of HeLa Kyoto cells following transfection with an RNAi molecule targeting endogenous PARP16 (SEQ ID NO: 43) following staining with an anti-tubulin antibody, Alexa Fluor 594 or 488 antibody (Invitrogen), and DAPI.

FIG. 11 is a set of micrographs showing the co-localization of PARP7-GFP and coilin, and the co-localization of PARP16-GFP and calnexin. In each experiment, HeLa Kyoto cells transfected with pEGFP-C1 vectors expressing PARP7-GFP or PARP16-GFP were stained with anti-GFP and anti-coilin or anti-calnexin antibodies, and fluorescently labeled secondary antibodies (Alexa Fluor 594 or 488 antibodies). The figure also lists a number of protein markers of specific cellular organelles and structures.

FIG. 12 is a diagram of the pcDNA3.1 vector (Invitrogen) showing the CMV promoter and the restriction sites that may be used for cloning.

FIG. 13 is a diagram of an example of an activity assay using one or more of the PARP-GFP fusion proteins of the invention.

FIG. 14 is a diagram of an example of an assay for identifying an activator of one or more PARP-GFP fusion proteins of the invention.

FIG. 15 is a picture of the Bio-Gel P-6 structure and a picture of a Coomassie Blue-stained SDS-PAGE gel showing the use of Bio-Gel P-6 for the purification of proteins from a crude HeLa Kyoto cell extract. The SDS-PAGE gel shows the proteins present in cell extract (Extract), in cell extract following lectin clarification (Lectin Clarification), in the lysate prior to passing over the Bio-Gel P-6 resin (Input), in the pellet following centrifugation of the resin (Pellet), and in the eluate following treatment with poly-ADP-ribose glycohydrolase ARH3 (ARH3 Release).

FIG. 16 is a set of micrographs showing the co-localization of poly-ADP ribose polymers (pADPR) and eIF3, a part of the translation initiation complex and a marker of stress granules, in HeLa Kyoto cells following treatment with 0 or 250 μ M sodium arsenite for 30 minutes and immunostaining with primary antibodies specific for poly-ADP-ribose polymers and eIF3, and Alexa Fluor 594 or 488 secondary antibodies (Invitrogen).

FIG. 17 is a set of micrographs showing the co-localization of PARP-GFP fusion proteins with eIF3 in transfected HeLa Kyoto cells following treatment with 0 or 250 μ M sodium arsenite for 30 minutes. In these experiments, HeLa Kyoto cells were transfected with a pEGFP-C1 plasmid expressing PARP5A-GFP, PARP12-GFP, PARP13.1-GFP, PARP13.2-GFP, or PARP15-GFP fusion protein and treated with 0 or 250 μ M sodium arsenite. The cells were fixed and stained with

anti-GFP and anti-eIF3, and secondary fluorescently-labeled antibodies (Alexa Fluor 594 or 488 antibodies (Invitrogen)) prior to imaging.

FIG. 18 is a set of micrographs showing the co-localization of endogenous PARP5A, PARP12, PARP13/13.1, PARP15, or poly-ADP-ribose glycohydrolase (PARG), and eIF3 (a stress granule marker) in HeLa Kyoto cells following treatment with 250 μ M sodium arsenite for 30 minutes. In these experiments, cells were stained with rabbit antibodies specific for one of PARP5A, PARP12, PARP13/13.1, PARP15, or PARG, and an anti-eIF3 antibody, and fluorescently-labeled secondary antibodies (Alexa Fluor 594 or 488 antibodies (Invitrogen)).

FIG. 19 is a set of micrographs showing the localization of poly-ADP-ribose (PAR), endogenous PARP5A, PARP12, PARP13, and PARP15, and eIF3 (a stress granule marker) in hTERT RPE cells following treatment with 250 μ M sodium arsenite for 30 minutes. In these experiments, cells were stained with antibodies specific for one of PAR, PARP5A, PARP12, PARP13, or PARP15, or an anti-eIF3 antibody, and a secondary fluorescently-labeled antibodies (Alexa Fluor 594 or 488 antibodies (Invitrogen)).

FIG. 20 is a set of micrographs showing the effect of PARP13.1-GFP, PARP13.2-GFP, or PARP15-GFP overexpression on stress granule formation. In these experiments, HeLa Kyoto cells were transfected with a plasmid expressing PARP13.1-GFP, PARP13.2-GFP, or PARP15-GFP. The cells were fixed and stained using rabbit anti-GFP and anti-eIF3 antibodies, and fluorescently-labeled secondary antibodies (Alexa Fluor 594 or 488 antibodies (Invitrogen)). The cells were also co-stained with DAPI.

FIG. 21 is a set of micrographs showing the co-localization of PARP11-GFP and eIF3 (a stress granule marker) in HeLa Kyoto cells transfected with a pEGFP-C1 vector expressing PARP11-GFP following treatment with 250 μ M sodium arsenite for 30 minutes. Following arsenite treatment, the cells were immediately fixed and stained using rabbit anti-GFP and anti-eIF3 antibodies, and fluorescently-labeled secondary antibodies (Alexa Fluor 594 or 488 antibodies (Invitrogen)). The cells were also stained with DAPI.

FIG. 22 is a set of micrographs showing the effect of PARG99-GFP, PARG102-GFP, or PARG110-GFP overexpression on stress granule formation in HeLa Kyoto cells transfected with a pEGFP-C1 plasmid containing a nucleic acid sequence encoding each PARG-GFP fusion protein, following treatment with 100 μ M sodium arsenite for 30 minutes. Following arsenite treatment, the cells were fixed and stained with rabbit anti-GFP and anti-eIF3 antibodies, and fluorescently-labeled secondary antibodies (Alexa Fluor 594 or 488 antibodies (Invitrogen)). The images shown in the right panels show the same data using a threshold filter.

FIG. 23 is a set of micrographs showing the effect of PARG or ARH3 knockdown on stress granule formation in HeLa Kyoto cells transfected with 30 nM PARG siRNA (CCAG-UUGGAUGGACACUAAUU (SEQ ID NO: 34) and UAC-GAAGGUACC AUAGAAUU (SEQ ID NO: 35)), ARH3 siRNA (GGACAGAAGCCUUGUACUAAUU (SEQ ID NO: 36) and CCAUUGCUGGUGCCUACUAAUU (SEQ ID NO: 37)), or a control siRNA (All Stars Negative Control siRNA; Qiagen Catalog No. 1027280) following treatment with 100 μ M sodium arsenite for 30 minutes, or 30 minutes or 1 hour following sodium arsenite washout. The cells were fixed and stained with an anti-eIF3 antibody and secondary fluorescently-labeled antibodies (Alexa Fluor 594 or 488 antibodies) to visualize stress granule formation. The panel on the left shows an immunoblot of cell lysate from HeLa Kyoto cells

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treated with 30 nM PARG siRNA, ARH3 siRNA, or control siRNA for 48 hours. The immunoblot was developed using an anti-PARG antibody.

FIG. 24 is a graph showing the percentage of HeLa Kyoto cells transfected with 30 nM PARG siRNA (SEQ ID NOS: 34 and 35), ARH3 siRNA (SEQ ID NOS: 36 and 37), or a control siRNA (All Stars Negative Control siRNA; Qiagen Catalog No. 1027280) containing stress granules following treatment with 100 μ M sodium arsenite for 30 minutes, or 30 minutes or 1 hour following sodium arsenite washout. The cells were fixed and stained with a fluorescently-labeled anti-eIF3 antibody to visualize stress granule formation.

FIG. 25A is a Silver-stained 4-12% SDS-PAGE gel showing the proteins immunoprecipitated with an anti-GFP antibody from lysate from HeLa S3 cells transfected with a pEGFP-C1 plasmid expressing GFP alone, PARP5A-GFP, PARP12-GFP, PARP13-GFP, PARP13.1-GFP, or PARP15-GFP following treatment with 0 or 250 μ M sodium arsenite for 30 minutes.

FIG. 25B is picture of an immunoblot of a 4-12% SDS-PAGE gel containing proteins immunoprecipitated with an anti-GFP antibody from lysate from HeLa S3 cells transfected with a pEGFP-C1 plasmid expressing GFP, PARP5A-GFP, PARP12-GFP, PARP13-GFP, PARP13.1-GFP, or PARP15-GFP following treatment with 0 or 250 μ M sodium arsenite for 30 minutes. The immunoblot was developed using a polyclonal anti-poly-ADP-ribose antibody.

FIG. 25C is a picture of several immunoblots of a 4-12% SDS-PAGE gel containing proteins immunoprecipitated with an anti-GFP antibody from lysate from HeLa S3 cells transfected with a pEGFP-C1 plasmid expressing GFP, PARP5A-GFP, PARP12-GFP, PARP13-GFP, PARP13.1-GFP, or PARP15-GFP following treatment with 0 or 20 nM pateamine A for 30 minutes. The immunoblots were developed using one of the following antibodies: anti-Ago2, anti-DDX6, anti-LSM1, anti-PABP, anti-FMRP, anti-eIF1A, anti-eIF2 α , anti-eIF3 η , anti-eIF4A1, and anti-eIF4E.

FIG. 26A is picture of an immunoblot of a 4-12% SDS-PAGE gel containing proteins immunoprecipitated with an anti-GFP antibody from lysate from HeLa S3 cells transfected with a pEGFP-C1 plasmid expressing TIA1-GFP, PABP-GFP, G3BP-GFP, or Ago2-GFP following treatment with 0 or 20 nM pateamine A for 30 minutes. The immunoblot was developed using a polyclonal anti-poly-ADP-ribose antibody.

FIG. 26B is a picture of an immunoblot of an SDS-PAGE gel containing proteins immunoprecipitated from lysate from untransfected HeLa S3 cells using anti-G3BP and anti-Ago2 antibodies following treatment with 0 or 250 μ M sodium arsenite for 60 minutes. The immunoblot was developed using a polyclonal anti-poly-ADP-ribose antibody.

FIG. 26C is a picture of an immunoblot of an SDS-PAGE gel containing proteins immunoprecipitated from lysate from HeLa S3 cells transfected with a pEGFP-C1 plasmid expressing G3BP1-GFP (full-length), G3BP1-A-GFP (domain A), G3BP1-ABC-GFP (domains A, B, and C), G3BP1-BC-GFP (domains B and C), G3BP1-BCD-GFP (domains B, C, and D), and G3BP1-D-GFP (domain D) following treatment with 0 or 250 μ M sodium arsenite for 60 minutes. The immunoblot was developed using a polyclonal anti-poly-ADP-ribose antibody.

FIG. 26D is a picture of an immunoblot of an SDS-PAGE gel containing proteins immunoprecipitated from lysate from HeLa S3 cells transfected with a pEGFP-C1 plasmid expressing TIA1-GFP (full-length) or TIA1 Δ RRM (mutant lacking RRM domain) following treatment with 0 or 250 μ M sodium

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arsenite for 60 minutes. The immunoblot was developed using a polyclonal anti-poly-ADP-ribose antibody.

FIG. 27 (left panel) is a set of micrographs showing the localization of poly-ADP-ribose, and endogenous Ago2 and eIF3 in HeLa cells following treatment with 250 μ M sodium arsenite for 30 minutes. The cells were imaged using fluorescently labeled anti-poly-ADP-ribose, anti-Ago2, and anti-eIF3 antibodies. FIG. 26 (right panel) is an immunoblot of a 4-12% SDS-PAGE gel containing proteins immunoprecipitated using an anti-Ago2 antibody from untransfected HeLa cells following treatment with 0 or 250 μ M sodium arsenite for 60 minutes. The immunoblot was developed using anti-poly-ADP-ribose antibodies.

FIG. 28 is a picture of an immunoblot of a 4-12% SDS-PAGE gel containing proteins immunoprecipitated with an anti-Ago2 antibody from lysate from untransfected HeLa cells following treatment with 0 or 250 μ M sodium arsenite for 30 minutes. The immunoblot was developed using a polyclonal anti-PARP13/13.1 antibody.

FIG. 29 is a picture of a Coomassie Blue-stained 4-12% SDS-PAGE gel containing proteins immunoprecipitated using an anti-GFP antibody from lysate from HeLa S3 cells transfected with a pEGFP-C1 plasmid expressing PARP13-GFP following treatment with 0 or 250 μ M sodium arsenite for 30 minutes.

FIG. 30 is a graph showing the relative expression of luciferase in lysates from 293T cells transfected with a modified pGL4.72[hRlucCP]TM vector (Promega); 10 nM of vector-target RNAi (SEQ ID NOS: 38 and 39); and a pEGFP-C1 vector encoding EGFP, G3BP, PAPR5A, PARP12, PARP13, PARP13.1, or PARP15. Luciferase expression was measured in cell lysates at 48 hours post-transfection. The level of luciferase in treated cells is compared to the level of luciferase produced in cells transfected with the modified pGL4.72 [hRlucCP]TM vector alone. As another positive control, the level of luciferase produced from cells transfected with the modified pGL4.72[hRlucCP]TM vector and the vector-target RNAi is shown (CXCR4 sponge).

FIG. 31 is a graph showing the relative expression of luciferase in 293T cells transfected with a modified pGL4.72 [hRlucCP]TM vector and 20 nM of negative RNAi control for PARP13 siRNA (siNeg; All Stars Negative Control siRNA; Qiagen Catalog No. 1027280) or PARP13 siRNA (si-PARP13; GCUCACGGAACUAUGAGCUGAGUUU; SEQ ID NO: 40) following treatment with 0 or 30 nM pateamine A for 30 minutes. Luciferase expression was measured in cell lysates at 48 hours post-transfection.

DETAILED DESCRIPTION

We have discovered that specific PARP proteins and subsets of PARP proteins have an effect on stress granule formation, nucleation, or disassembly, and/or are localized in the nucleus and are required for cell cycle progression through mitosis. We have also discovered that PARGs have an effect on stress granule formation, nucleation, and disassembly (i.e., stress granule kinetics). The invention therefore provides methods, compositions, and kits for the treatment of stress granule-related disorders and cancer, and methods for determining the propensity of a subject to develop a stress granule-related disorder or cancer based on these unique activities of specific PARP proteins and PARGs. Conversely, the invention also provides methods for increasing the proliferation rate of a cell or cell population and increasing stress granule formation in a cell or cell population by modulating PARP function, PARG function, or poly-ADP-ribose pathways or homeostasis in a cell or cell population. Lastly, the

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invention provides screening methods for the identification of candidate agents that may be useful for treating or reducing the likelihood of developing a stress granule-related disorder and/or cancer.

Methods for Treating a Stress-Granule Related Disorder and Decreasing Stress Granule Formation

The present invention provides methods for treating or reducing the likelihood of developing one or more stress-granule related disorders in a subject. Stress granule-related disorders share a common etiology and pathology linked to oxidative stress and inflammation. Stress granule-related disorders include, without limitation, cardiovascular disorders, inflammatory disorders, neurological disorders, or ischemic-reperfusion injury.

In the treatment methods provided by the invention, a subject is administered a therapeutically effective dose of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARP inhibitor(s), one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARG activator(s), and/or one or more (e.g., 1, 2, 3, 4, or 5) PARP11 activator(s). A patient receiving the treatment may be previously diagnosed as having a stress granule-related disorder, or may be diagnosed as having a high probability (i.e., at significant risk) of developing a stress granule-related disorder. A person receiving the treatment may be asymptomatic or may be experiencing one or more (e.g., 1, 2, 3, 4, or 5) of the symptoms of a stress granule-related disorder.

Non-limiting examples of stress granule related disorders include an aneurysm, angina, atherosclerosis, stroke, cerebrovascular disease, congestive heart failure, coronary artery disease, myocardial disease, peripheral vascular disease, granulomatous myocarditis, chronic myocarditis, myocardial infarction, primary hypertrophic cardiomyopathy, autoimmune diseases, asthma, allergic intraocular inflammatory diseases, arthritis, atopic dermatitis, atopic eczema, cirrhosis, Crohn's disease, ulcerative colitis, diabetes, hemolytic anemia, inflammatory dermatosis, an inflammatory bowel disorder, systemic lupus erythematosus, psoriasis, rheumatoid arthritis, Wegener's granulomatosis, Hashimoto's thyroiditis, chronic pancreatitis, reactive lymphoid hyperplasia, multiple sclerosis, Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, retinosa pigmentosa, macular degeneration, traumatic brain injury, stroke, and peripheral neuropathy. Methods for the diagnosis and monitoring of several stress granule related disorders and known in the art. The methods of the invention may reduce (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100%) one or more (e.g., 1, 2, 3, 4, or 5) of the symptoms of a stress granule related-disorder or prevent the onset of one or more (e.g., 1, 2, 3, 4, or 5) of the symptoms of a stress-related disorder. Symptoms of a stress granule-related disorder include, but are not limited to, pain, swelling, inflammation, loss of cognition, loss of vision, loss of coordination, difficulty breathing, airway constriction, artery occlusion, diarrhea, elevated blood glucose, increased levels of pro-inflammatory cytokines, increase protein aggregates or deposits, and increased cell death (e.g., apoptosis or necrosis). The methods of treatment provided herein may be used with one or more (e.g., 1, 2, 3, 4, or 5) other therapies or therapeutic agents used to treat a stress granule-related disorder. The effectiveness of treatment may be measured by a physician using methods known in the art.

In the treatment methods provided herein, a subject may be administered one or more (e.g., 1, 2, 3, 4, or 5) PARP inhibitors. The one or more PARP inhibitors preferably decrease (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100% decrease) the expression (e.g., protein and/or mRNA levels) and/or one or more activities of

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PARP5A, PARP12, PARP13.1, PARP13.2, and PARP15. The decrease in expression of PARP5A, PARP12, PARP13.1, PARP13.2, and PARP15 preferably is a decrease in the expression of one or more nucleic acids containing a sequence having at least 80% sequence identity (e.g., 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100% identity) to PARP5A (SEQ ID NO: 8 or 9), PARP5B (SEQ ID NO: 10), PARP13.1 (SEQ ID NO: 19), PARP13.2 (SEQ ID NO: 20), or PARP15 (SEQ ID NO: 22 or 23), or one or more polypeptides encoded by these nucleic acids. The PARP inhibitors also preferably inhibit one or more of the activities of a PARP protein (e.g., PARP5A, PARP12, PARP13.1, PARP13.2, or PARP15) including poly-ADP-ribosylation of a target protein (e.g., a protein in a stress granule, a polypeptide involved in the formation or disassembly of a stress granule, or a PARP protein) or the formation or nucleation of a stress granule. Methods for measuring the activity of one or more PARP proteins are described herein.

A subject may also be administered one or more (e.g., 1, 2, 3, 4, or 5) PARG activators. Preferred PARG activators selectively increase the expression and/or biological activity of PARG protein or ARH3. For example, PARG activators desirably increase the level of one or more nucleic acid(s) containing a nucleic acid sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100% identity) to PARG (SEQ ID NO: 42) or ARH3 (SEQ ID NO: 41), or increase the level of one or more polypeptides encoded by these nucleic acids. Desirably, a PARG activator increases the one or more activities of a PARG protein or ARH3, including, but not limited to, hydrolysis of poly-ADP-ribose (e.g., poly-ADP-ribose attached to a substrate protein, e.g., a protein localized in a stress granule, a polypeptide involved in the formation or disassembly of a stress granule, or a PARP protein), the prevention of the assembly of a stress granule, or disassembly of a stress granule.

A subject may also be administered one or more (e.g., 1, 2, 3, 4, or 5) PARP11 activators. Desirably, the one or more PARP11 activators selectively increase the level of one or more nucleic acids containing a sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100% identity) to PARP11 (SEQ ID NO: 17), or increase the level of one or more polypeptides encoded by these nucleic acids. Additional desirable PARP11 activators increase one or more activities of PARP11, including, but not limited to, poly-ADP-ribosylation of a target protein (e.g., a protein localized in a stress granule or a polypeptide involved in the formation, a polypeptide involved in the disassembly of a stress granule, and/or a PARP protein), the prevention of the assembly of a stress granule, or the disassembly of a stress granule.

Examples of PARP inhibitors that may be used in these methods include an antibody or antibody fragment that selectively binds one or more of PARP5A, PARP12, PARP13.1, PARP13.2, and PARP15; an RNA aptamer (e.g., SEQ ID NOS: 40, 49, 99-113, and 122-129); or a small molecule. Examples of PARG activators that may be used in these methods include one or more nucleic acids containing a sequence having at least 80% sequence identity (e.g., 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100% identity) to PARG (SEQ ID NO: 42) or ARH3 (SEQ ID NO: 41). Examples of PARP11 activators that may be used in these methods include one or more nucleic acids containing a nucleic acid sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100% identity) to PARP11 (SEQ ID NO: 17).

In these methods, the one or more PARP inhibitors, one or more PARG activators, and one or more PARP11 activators may be administered co-extensively (overlapping bioactive periods) or non-extensively (non-overlapping bioactive periods). In one example, the one or more PARP inhibitors, one or more PARG activators, and one or more PARP11 activators may be administered together in a single dose or may be administered separately in one or more separate doses (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 doses). The amount of one or more PARP inhibitors, one or more PARG activators, and one or more PARP11 activators that may be administered to a subject per dose may be between 0.1 mg and 1 g, 0.1 mg and 750 mg, 0.1 mg and 600 mg, 0.1 mg and 500 mg, 10 mg and 450 mg, 10 mg and 400 mg, 10 mg and 350 mg, 10 mg and 350 mg, and 10 mg and 250 mg. Various combinations of the one or more PARP inhibitors, one or more PARG activators, and one or more PARP11 activators are contemplated herein, for example, administration of one or more PARP inhibitors alone, administration of one or more PARG activators alone, administration of one or more PARP11 activators alone, administration of one or more PARP inhibitors and one or more PARG activators together, and administration of one or more PARP inhibitors and one or more PARP11 activators together.

The one or more PARP inhibitors, one or more PARG activators, and the one or more PARP11 activators may be administered to the subjects once a day, twice a day, three times a day, once a week, twice a week, three times a week, four times a week, five times a week, six times a week, seven times a week, bi-weekly, tri-weekly, or monthly. The one or more PARP inhibitors, one or more PARG activators, and the one or more PARP11 activators may be administered via the same route of administration or via different routes of administration. For example, the one or more PARP inhibitors may be administered orally and the PARG activators may be administered parenterally (e.g., subcutaneously). The one or more PARP inhibitors, one or more PARG activators, and/or the one or more PARP11 activators may be formulated for any known route of administration, including oral, intravenous, intraarterial, intraocular, intranasal, intramuscular, and subcutaneous administration. The one or more PARP inhibitors, one or more PARG activators, and/or the one or more PARP11 activators may also be formulated for administration in a sustained-release form. The therapeutically effective dose of the one or more PARP inhibitors, one or more PARG activators, and/or one or more PARP11 activators may be determined by a skilled physician using methods known in the art, in addition to the in vitro assays described herein.

The invention similarly provides methods of decreasing the number of stress granules in a cell or a cell population by contacting the cell or cell population with an effective amount of one or more PARP inhibitors, one or more PARG activators, and one or more PARP11 activators (as described for the treatment of subjects above). In these methods, the one or more PARP inhibitors, one or more PARG activators, and the one or more PARP11 activators are added to the tissue culture medium to decrease the formation of stress granules in a cell or a cell population. Desirably, the cells are cultured for regenerative cell technology or the cells are primary or germ cells. This method desirably confers on the cells protection against oxidative stress and promotes the longevity and morphology of the cultured cells. Preferred cells that may be used in these methods include, without limitation, an epithelial cell, a fibroblast, a kidney cell, a muscle cell, a neuron, a hepatocyte, a sperm, a lymphocyte, or a macrophage. The concentration of the one or more PARP inhibitors, the one or more PARG activators, and the one or more PARP11 activa-

tors to be added to the culture medium may be determined by using the methods described herein (e.g., methods for the measurement of stress granule formation).

Methods for Treating a Cancer and Decreasing Cell Proliferation

The present invention provides methods for treating or reducing the likelihood of developing cancer in a subject. All forms of cancer share a common etiology and pathology of uncontrolled, unregulated, or misregulated cell proliferation or cell division. Cancers that may be treated by the methods, compositions, and kits of the invention include, without limitation, colon adenocarcinoma, esophagus adenocarcinoma, liver hepatocellular carcinoma, squamous cell carcinoma, pancreas adenocarcinoma, islet cell tumor, rectum adenocarcinoma, gastrointestinal stromal tumor, stomach adenocarcinoma, adrenal cortical carcinoma, follicular carcinoma, papillary carcinoma, breast cancer, ductal carcinoma, lobular carcinoma, intraductal carcinoma, mucinous carcinoma, phyllodes tumor, Ewing's sarcoma, ovarian adenocarcinoma, endometrium adenocarcinoma, granulose cell tumor, mucinous cystadenocarcinoma, cervix adenocarcinoma, vulva squamous cell carcinoma, basal cell carcinoma, prostate adenocarcinoma, giant cell tumor of bone, bone osteosarcoma, larynx carcinoma, lung adenocarcinoma, kidney carcinoma, urinary bladder carcinoma, Wilm's tumor, lymphoma, and non-Hodgkin's lymphoma. The methods of the invention may reduce (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100%) one or more (e.g., 1, 2, 3, 4, or 5) of the symptoms of a cancer or prevent the onset of one or more (e.g., 1, 2, 3, 4, or 5) of the symptoms of a cancer. Symptoms of a cancer include, but are not limited to, blood in urine, pain or burning upon urination, cloudy urine, pain in bone, fractures in bones, fatigue, weight loss, repeated infections, nausea, vomiting, constipation, numbness in the legs, bruising, dizziness, drowsiness, abnormal eye movements, changes in vision, changes in speech, headaches, thickening of a tissue, rectal bleeding, abdominal cramps, loss of appetite, fever, enlarged lymphnodes, persistent cough, blood in sputum, lung congestion, itchy skin, lumps in skin, abdominal swelling, vaginal bleeding, jaundice, heartburn, indigestion, cell proliferation, and loss of regulation of controlled cell death. The methods of treatment provided herein may be used with one or more (e.g., 1, 2, 3, 4, or 5) other therapies or therapeutic agents used to treat a cancer (e.g., chemotherapy, radiation, and/or surgery). The effectiveness of treatment may be measured by a physician using methods known in the art. Methods for the diagnosis and monitoring of several cancers are known in the art.

In the treatment methods provided by the invention, a subject is administered a therapeutically effective dose of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) of a PARP inhibitor(s). A patient receiving the treatment may be previously diagnosed as having a cancer, or may be diagnosed as having a high probability (i.e., at significant risk) of developing a cancer. A person receiving the treatment may be asymptomatic or may be experiencing one or more of the symptoms of a cancer.

In the treatment methods provided herein, a subject may be administered one or more PARP inhibitors. The one or more PARP inhibitors preferably decrease (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100% decrease) the expression and/or one or more (e.g., 1, 2, 3, 4, or 5) activities of one or more of PARP1, PARP2, PARP5A, PARP5B, PARP7, PARP8, PARP14, or PARP16. Preferably, the decrease in expression is a decrease in the level of one or more nucleic acids containing a sequence having at least 80% sequence identity (e.g., 85%, 90%, 95%, 96%, 97%, 98%,

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99%, or even 100% identity) to PARP1 (SEQ ID NO: 1 or 2), PARP2 (SEQ ID NO: 3), PARP5A (SEQ ID NO: 8 or 9), PARP5B (SEQ ID NO: 10), PARP7 (SEQ ID NO: 12), PARP8 (SEQ ID NO: 13), PARP14 (SEQ ID NO: 21), or PARP16 (SEQ ID NO: 24), or one or more polypeptides encoded by these nucleic acids. The PARP inhibitors also preferably inhibit one or more of the activities of a PARP protein (e.g., PARP1, PARP2, PARP5A, PARP5B, PARP7, PARP8, PARP14, or PARP16) including poly-ADP-ribosylation of a target protein (e.g., a protein localized in the nucleus or mitotic spindle during cytokinesis, a protein required for progression through mitosis, and/or a PARP protein) or is required for progression through mitosis. Methods for measuring the activity of one or more PARP proteins are described herein.

Examples of PARP inhibitors that may be used in these methods include an antibody or antibody fragment that selectively binds one or more of PARP1, PARP2, PARP5A, PARP5B, PARP7, PARP8, PARP14, and PARP16; an RNA aptamer (e.g., SEQ ID NOS: 43-46, 49, 50, 59-74, 114-121, and 130-136; shown in table below); or a small molecule. In these methods, the one or more PARP inhibitors may be administered co-extensively (overlapping bioactive periods) or non-extensively (non-overlapping bioactive periods). In one example, the one or more PARP inhibitors may be administered together in a single dose or may be administered separately in one or more separate doses (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 doses). The amount of one or more PARP inhibitors may be between 0.1 mg and 1 g, 0.1 mg and 750 mg, 0.1 mg and 600 mg, 0.1 mg and 500 mg, 10 mg and 450 mg, 10 mg and 400 mg, 10 mg and 350 mg, 10 mg and 350 mg, and 10 mg and 250 mg. Various combinations of the one or more PARP inhibitors are contemplated herein, for example, administration of one or more PARP inhibitors alone or co-administration of one or more PARP inhibitors with another therapeutic for the treatment of cancer or a therapeutic used to alleviate one or more symptoms of a cancer.

TABLE 1

Specific PARP RNAi molecules		
PARP1	AAGCCUCCGCUCCUGAACAAU	SEQ ID NO: 44
PARP2A	AAUCAGUGUAAUGAACUACUA	SEQ ID NO: 45
PARP2B	AAUGAUUCAGCUAUUAGAAGA	SEQ ID NO: 46
PARP3	GGACCCAGGUGUAUGAGGACUACAA	SEQ ID NO: 47
PARP4	AAACAAGGAUUUCUACUAAGA	SEQ ID NO: 48
PARP5A	AACAAUUCACCGUCGUCCUCU	SEQ ID NO: 49
PARP5B	AAGCUUCAGAAUGGUGCAAAU	SEQ ID NO: 50
PARP6	CCCAACAAUGGAAACAUUCUGAGCAA	SEQ ID NO: 51
PARP6	UUGCUCAGAUGUUUCCAUUGUUGG	SEQ ID NO: 52
PARP6	GGUUAAGGCAAGUGGUACCAUCAA	SEQ ID NO: 53
PARP6	UUGAUGGUACACUUGCCUUGAACC	SEQ ID NO: 54
PARP6	CAAAGUGGAAGUGUUUGGCUACCCU	SEQ ID NO: 55
PARP6	AGGGUAGCCAAACACUCCACUUUG	SEQ ID NO: 56
PARP6	CAGAACAGAGGAUUCACAAUUGAA	SEQ ID NO: 57
PARP6	UUCAAUGUUGGAUCCUCUGUUCUG	SEQ ID NO: 58

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TABLE 1-continued

Specific PARP RNAi molecules		
PARP7	UGAGGUCUUUGAGGCCAAUUAUAAA	SEQ ID NO: 59
PARP7	UUUAAUAUUGGCCUCAAAGACCUCA	SEQ ID NO: 60
PARP7	GACUUUCUGCAAGGCACUUGUAUUU	SEQ ID NO: 61
PARP7	AAAUACAAGUGCCUUGCAGAAAGUC	SEQ ID NO: 62
PARP7	UCCUCCACCUCUUGAAGCAACUUCA	SEQ ID NO: 63
PARP7	UGAAGUUGCUUCAAGAGGUGGAGGA	SEQ ID NO: 64
PARP7	AAUGAUGACCAGAGUUACCCUUAUU	SEQ ID NO: 65
PARP7	AAUAAGGGUAAACUCUGGUCAUCAU	SEQ ID NO: 66
PARP8	GGAAGAUAUCUGAAGGUGACAAUGAU	SEQ ID NO: 67
PARP8	AUCAUUGUCACCUUCAGAAUUCUCC	SEQ ID NO: 68
PARP8	CCCACAACUGGAAGCUGAUUUGUCA	SEQ ID NO: 69
PARP8	UGACAAAUCAGCUUCCAGUUGUGGG	SEQ ID NO: 70
PARP8	GAAGUGGAUUCUAUCUUGUCCAAU	SEQ ID NO: 71
PARP8	AUUGGACUAAGAUAGAUAUCCACUUC	SEQ ID NO: 72
PARP8	GCCUUAUGUGAAGUGAUCACCUCAU	SEQ ID NO: 73
PARP8	AUGAGGUGAUCACUUCACAUAGGC	SEQ ID NO: 74
PARP9	GCCGGAGCAGCAGCUUACAAUGAAA	SEQ ID NO: 75
PARP9	UUUCAUUGUAAGCUGCUGCCCGGC	SEQ ID NO: 76
PARP9	CCCUCUGAAUUUGUGUACAAGACU	SEQ ID NO: 77
PARP9	AGUCUUUGUACACAAUUCAGAGGG	SEQ ID NO: 78
PARP9	GGACCCUACUGUUGCUGCCUUUAAA	SEQ ID NO: 79
PARP9	UUUAAAGGCAGCAACAGUAGGGUCC	SEQ ID NO: 80
PARP9	UGGCAGACGGCAGAUUAUUGUUA	SEQ ID NO: 81
PARP9	UAACAAUUAUACUUGCCGUCUGCCA	SEQ ID NO: 82
PARP10	CAUGGUGCAGGGUAGAGGGAUUUUG	SEQ ID NO: 83
PARP10	CAUAAUCCCUUACCCUGCACCAUG	SEQ ID NO: 84
PARP10	GCCUGUGGAGAUUGGUGCUAUUGAU	SEQ ID NO: 85
PARP10	AUCAAUAGCACCAUCCACCAGGC	SEQ ID NO: 86
PARP10	AGACGUCGUCUCUUGCCACUUGAA	SEQ ID NO: 87
PARP10	UUCAAGUGGCAAGAGAGCGACGUCU	SEQ ID NO: 88
PARP10	UGGGCAGCAUAGCUGCCAUGUGUU	SEQ ID NO: 89
PARP10	AACACAUGGCAGCUAAUGCUGCCCA	SEQ ID NO: 90
PARP11	CAACAAACAAUGAAGUGGAUGACAU	SEQ ID NO: 91
PARP11	AUGUCAUCCACUUAUUGUUUGUUG	SEQ ID NO: 92
PARP11	CAGCCGGAUACCAACAGUCAGUGUU	SEQ ID NO: 93
PARP11	AACACUGACUGUUGGUAUCCGGCUG	SEQ ID NO: 94
PARP11	CAAACCCUUGUGGCUCCAUUUCUUU	SEQ ID NO: 95
PARP11	AAAGAAAUGGAGCCACAAGGUUUG	SEQ ID NO: 96
PARP11	UGCCACCACACUGGGAGAAUGUGAA	SEQ ID NO: 97

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TABLE 1-continued

Specific PARP RNAi molecules		
PARP11	UUCACAUUCUCCAGUGUGGUGCA	SEQ ID NO: 98
PARP12	UCCACCUCUGCAGGUCAUGGUCUA	SEQ ID NO: 99
PARP12	UAGACCAUGAACCUGCAGAGGUGGA	SEQ ID NO: 100
PARP12	UGCCAGAAAUUGCCAACAUUACAA	SEQ ID NO: 101
PARP12	UUGUAAUGUUGGCAAAUUCUGGCA	SEQ ID NO: 102
PARP12	GGUGAGCAGGCUGCCUACCAUUUAU	SEQ ID NO: 103
PARP12	AUAAAUGGUAGGCAGCCUGCUCACC	SEQ ID NO: 104
PARP12	AGGAUUGGACAACAUGGAACUUAU	SEQ ID NO: 105
PARP12	AUAAGUUCUAGUUGUCCAAUCCU	SEQ ID NO: 106
PARP13	GCUGACCCAGAGUAGCACUUGUUA	SEQ ID NO: 107
PARP13	UAACAAGUGCUACUUGGGUGCAGC	SEQ ID NO: 108
PARP13	CCGGUGGCAGAUUGCUUUAUGGUA	SEQ ID NO: 109
PARP13	UUUACCAAUAAGCAUCUGCCACCGG	SEQ ID NO: 110
PARP13	GCUCACGGAACUAUGAGCUGAGUUU	SEQ ID NO: 40
PARP13	AAACUCAGCUCAUAGUUCUGGAGC	SEQ ID NO: 111
PARP13	UGCCUCAGUGGUAUGUGCAGCAGAU	SEQ ID NO: 112
PARP13	AUCUGCUGCACAUAACACUGAGGCA	SEQ ID NO: 113
PARP14	UGGCCUGUCUAAUGAUGACUUAU	SEQ ID NO: 114
PARP14	UUGAAAGUCAUCAUAGACAGGCCA	SEQ ID NO: 115
PARP14	CCUGGUGCUGAUGACUACGUUUA	SEQ ID NO: 116
PARP14	UUAAACUGUAGUCAUCAGCACCAGG	SEQ ID NO: 117
PARP14	GCCACUUUCUGUGUCCCAUACUAU	SEQ ID NO: 118
PARP14	AUAGUAUGGGAAACACAGAAUGGCG	SEQ ID NO: 119
PARP14	GAAGAGUCACUAGAUUCCCUUAU	SEQ ID NO: 120
PARP14	AUAAGGGAAGAUUAGUGACUCUUC	SEQ ID NO: 121
PARP15	GAUGAAUUCACUAACUGGUAAGAA	SEQ ID NO: 122
PARP15	UUCUUGACCAGUAGUAAUUAUC	SEQ ID NO: 123
PARP15	CCUAUCACAGUUGCUGAUAAUAUA	SEQ ID NO: 124
PARP15	UUAUGUUAUCAGCAACUGUGAUAGG	SEQ ID NO: 125
PARP15	GGACUGACAUGAAUUAUCAGCUGUU	SEQ ID NO: 126
PARP15	AACAGCUGAUGAUUAUGUCAGUCC	SEQ ID NO: 127
PARP15	CGAGUACUUAUCUGGAGUCUACAA	SEQ ID NO: 128
PARP15	UUGUGAAGACUCCAGUAAGUACUCG	SEQ ID NO: 129
PARP16	CAGUCAGGGAAGGCAGAGUUGAA	SEQ ID NO: 130
PARP16	UUCAAACUCUGCCUCCUGCAGUCG	SEQ ID NO: 131
PARP16	GAGACCAAAGGAGAACGAGACCUAA	SEQ ID NO: 132
PARP16	UUAGGUCUCGUUCUCCUUGGUCUC	SEQ ID NO: 133
PARP16	GACUUGAGCCUGGCCUCAUAUACA	SEQ ID NO: 134
PARP16	UGUAUUGAGGGCCAGGCUCAGUC	SEQ ID NO: 135

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TABLE 1-continued

Specific PARP RNAi molecules		
PARP16	CCCAAGUACUUCGUGGUCACCAUA	SEQ ID NO: 43
PARP16	UAUUGGUGACCACGAAGUACUUGG	SEQ ID NO: 136

The one or more PARP inhibitors may be administered to the subjects once a day, twice a day, three times a day, once a week, twice a week, three times a week, four times a week, five times a week, six times a week, seven times a week, bi-weekly, tri-weekly, or monthly. The one or more PARP inhibitors may be administered via the same route of administration or via different routes of administration. For example, the one or more PARP inhibitors may be administered orally and one or more other PARP inhibitors may be administered parenterally (e.g., subcutaneously). The one or more PARP inhibitors may be formulated for any known route of administration, including oral, intravenous, intraarterial, intraocular, intranasal, intramuscular, and subcutaneous administration. The one or more PARP inhibitors may also be formulated for administration in a sustained-release form.

The invention similarly provides methods of decreasing the proliferation rate in a cell or a cell population by contacting the cell or cell population with an effective amount of one or more PARP inhibitors (as described for the treatment of subjects above). In these methods, the one or more PARP inhibitors are added to the tissue culture medium to decrease the proliferation rate of a cell or a cell population. Desirably, the cells are cultured for regenerative cell technology, the cells are primary or germ cells, or the cells are being stored for later therapeutic or experimental use. This method desirably confers on the cells protection from oxidative stress, promotes the longevity and morphology of the cultured cells, and/or delays the onset of senescence of the cells. Preferred cells that may be used in these methods include, without limitation, an epithelial cell, a fibroblast, a kidney cell, a muscle cell, a neuron, a hepatocyte, a sperm, a lymphocyte, or a macrophage. The concentration of the one or more PARP inhibitors may be determined using methods known in the art and those methods described herein (e.g., methods for the measurement of cell growth and division).

Pharmaceutical Compositions

The invention further provides pharmaceutical compositions for treating or reducing the likelihood of developing one or more stress granule disorders and cancer. For example, the compositions for the treating or decreasing the likelihood of developing a stress granule-related disorder may include one or more of the PARP inhibitors (e.g., PARP5A, PARP12, PARP13.1, PARP13.2, and PARP15 inhibitors), one or more of the PARG activators (e.g., PARG protein and ARH3 activators), and/or one or more of the PARP11 activators as described above. Compositions for treating or decreasing the likelihood of developing a cancer may include one or more of the PARP inhibitors (e.g., PARP 1, PARP2, PARP5A, PARP5B, PARP7, PARP8, PARP14, and PARP16 inhibitors) as described above. The one or more agents that may be used as pharmaceutical compositions for treating or reducing the likelihood of developing a stress granule-related disorder or cancer may also be identified using the screening assays provided herein.

Examples of pharmaceutical compositions for treating or reducing the likelihood of developing a stress granule related disorder include one or more of an antibody or antibody fragment that specifically binds to PARP5A, PARP12,

PARP13.1, PARP13.2, and PARP15; an RNAi molecule that decreases the expression of one or more of PARP5A, PARP12, PARP13.1, PARP13.2, and PARP15 (e.g., SEQ ID NOS: 40, 49, 99-113, and 122-129); one or more nucleic acids containing a sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARP11 (SEQ ID NO: 17), PARG (SEQ ID NO: 42), or ARH3 (SEQ ID NO: 41), and one or more small molecules or metabolites identified in the screening assays provided herein.

Examples of pharmaceutical compositions for treating or reducing the likelihood of developing a cancer include one or more of an antibody or antibody fragment that specifically binds to PARP1, PARP2, PARP5A, PARP5B, PARP7, PARP8, PARP14, and PARP16; an RNAi molecule that decreases the expression of one or more of PARP1, PARP2, PARP5A, PARP5B, PARP7, PARP8, PARP14, and PARP16 (e.g., SEQ ID NOS: 43-46, 49, 50, 59-74, 114-121, and 130-136); and one or more small molecules or metabolites identified in the screening assays provided herein.

The pharmaceutical compositions provided by the invention may further include one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) secondary agents. Non-limiting examples of secondary agents that may be included in the compositions of the invention are one or more chemotherapeutic agent(s), one or more non-steroidal anti-inflammatory drug(s), one or more immunosuppressive agent(s), one or more calcineurin inhibitor(s), or one or more analgesic(s). Examples of these classes of therapeutic agents are known in the art.

The dose of one or more PARP inhibitors, PARP11 activators, and/or PARG activators may be between 0.1 mg and 1 g, 0.1 mg and 750 mg, 0.1 mg and 600 mg, 0.1 mg and 500 mg, 10 mg and 450 mg, 10 mg and 400 mg, 10 mg and 350 mg, 10 mg and 350 mg, and 10 mg and 250 mg.

The dose of one or more secondary agents that may be included in the compositions of the invention may be between 0.1 mg and 2 g, 0.1 mg and 1.5 mg, 0.1 mg and 1 g, 0.1 mg and 750 mg, 1 mg and 650 mg, 1 mg and 550 mg, 1 mg and 500 mg, 10 mg and 450 mg, 10 mg and 400 mg, 10 mg and 350 mg, 10 mg and 350 mg, and 10 mg and 250 mg.

The compositions may be formulated using any known method including formulation as a pill, an injectable fluid (e.g., in PBS), or in a sustained-release form. The compositions may be formulated for any specific route of administration including oral, intramuscular, intraocular, intranasal, subcutaneous, intraarterial, and intravenous administration. Kits

The invention further provides kits containing one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) of the pharmaceutical compositions described herein. The kits may further contain materials to aid in the administration of the pharmaceutical agents (e.g., a syringe). The kits may contain one or more doses of a pharmaceutical agent provided by the invention. The kits may further contain instructions for administering the pharmaceutical compositions to a subject having a stress granule-related disorder or cancer, or a subject that has a high probability of developing (a high propensity) for developing a stress granule-related disorder or cancer.

Medical Screening Assays

The invention further provides methods of determining the propensity of a subject to develop a stress granule-related disorder by determining the expression (e.g., protein or mRNA levels) and/or one or more (e.g., 1, 2, 3, 4, or 5) activities of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, or 8) of PARP5A, PARP11, PARP12, PARP13.1, PARP13.2, PARP15, PARG, and ARH3 in a subject, wherein an increase (e.g., at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%,

80%, 90%, or 100%) in the expression (e.g., mRNA and/or protein) and/or one or more (e.g., 1, 2, 3, 4, or 5) activities of one or more (e.g., 1, 2, 3, 4, or 5) of PARP5A, PARP12, PARP13.1, PARP13.2, and PARP15, and/or a decrease (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100%) in one or more (e.g., 1, 2, or 3) of PARG, ARH3, and PARP11 expression (e.g., protein or mRNA levels) and/or one or more activities indicates an increased propensity to develop a stress-granule related disorder. In this method, the expression measured may be the level of one or more nucleic acids containing a nucleic acid sequence having at least 80% (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARP5A (SEQ ID NO: 8 or 9), PARP5B (SEQ ID NO: 10), PARP11 (SEQ ID NO: 17), PARP13.1 (SEQ ID NO: 19), PARP13.2 (SEQ ID NO: 20), PARP15 (SEQ ID NO: 22 or 23), PARG (SEQ ID NO: 42), or ARH3 (SEQ ID NO: 41), or one or more polypeptides encoded by these nucleic acids.

The invention further provides methods of determining the propensity of a subject to develop a cancer by determining the expression (e.g., protein and/or mRNA level) and/or one or more activities (e.g., 1, 2, 3, 4, or 5) of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, or 8) of PARP1, PARP2, PARP5A, PARP5B, PARP7, PARP8, PARP14, and PARP16 in a subject, wherein an increase (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100%) in the expression and/or one or more activities of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, or 8) of PARP1, PARP2, PARP5A, PARP5B, PARP7, PARP8, PARP14, and PARP16 indicates an increased propensity to develop cancer. In this method, the expression measured may be the level of one or more nucleic acids containing a nucleic acid sequence having at least 80% (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) identity to PARP1 (SEQ ID NO: 1 or 2), PARP2 (SEQ ID NO: 3), PARP5A (SEQ ID NO: 8 or 9), PARP5B (SEQ ID NO: 10), PARP 7 (SEQ ID NO: 12), PARP8 (SEQ ID NO: 13), PARP14 (SEQ ID NO: 21), and PARP16 (SEQ ID NO: 16), or the level of one or more polypeptides encoded by these nucleic acids.

In each of these methods, the level of expression of a nucleic acid may be determined using RT-PCR and the level of a polypeptide encoded by a nucleic acid may be determined by a variety of different antibody-based techniques including, but not limited to, immunoblotting, fluorescence-assisted cell sorting (FACS), and enzyme-linked immunosorbent assay (ELISA). In each assay, the level of expression in a subject may be compared to a subject known to have a stress granule-related disorder or cancer, or a control subject that does not have a stress granule-related disorder or cancer, or a very low propensity for developing a stress granule-related disorder or cancer.

PARP, PARG, and ARH3 Fusion Proteins

General Design

The invention provides fusion proteins for each PARP, PARG, and ARH3. The fusion proteins may be used to identify unique biological activities for each PARP, PARG, and ARH3 protein and to identify specific inhibitors and activators for each PARP, PARG, or ARH3, or specific subsets of these proteins. The invention provides nucleic acid sequences encoding these PARP, PARG, and ARH3 fusion proteins. The nucleic acids contain a sequence that is at least 80% identical (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100% identical) to the full-length sequence of PARP1 (SEQ ID NO: 1 or 2), PARP2 (SEQ ID NO: 3), PARP3 (SEQ ID NO: 4), PARP3.2 (SEQ ID NO: 5), PARP3.3 (SEQ ID NO: 6), PARP4 (SEQ ID NO: 7), PARP5A (SEQ ID NO: 8 or 9), PARP5B (SEQ ID NO: 10), PARP6 (SEQ ID NO: 11), PARP7 (SEQ ID NO: 12), PARP8 (SEQ ID NO: 13), PARP9

(SEQ ID NO: 14), PARP10 (SEQ ID NO: 15), PARP10.2 (SEQ ID NO: 16), PARP11 (SEQ ID NO: 17), PARP12 (SEQ ID NO: 18), PARP13.1 (SEQ ID NO: 19), PARP13.2 (SEQ ID NO: 20), PARP14 (SEQ ID NO: 21), PARP15.1 (SEQ ID NO: 22), PARP15.2 (SEQ ID NO: 23), PARP16 (SEQ ID NO: 24), PARG (SEQ ID NO: 42), or ARH3 (SEQ ID NO: 41).

The nucleic acids of the invention further contain nucleic acid sequences encoding one or two polypeptide tags. The nucleic acids encoding a polypeptide tag may be placed at a position 5' or a position 3' to the sequence encoding a PARP, PARG, or ARH3 protein. For example, the 3' end of a nucleic acid sequence encoding a polypeptide tag may directly abut (i.e., no intervening nucleotides) the 5' end of a nucleic acid sequence encoding a PARP, PARG, or ARH3 protein. In another example, the 5' end of a nucleic acid sequence encoding a polypeptide tag may directly abut (i.e., no intervening nucleotides) the 3' end of nucleic acid sequence encoding a PARP, PARG, or ARH3 protein. In another example, one or more nucleotides (e.g., at least 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, or 400 nucleotides) separate the 5' end of the sequence encoding the polypeptide tag from the 3' end of the sequence encoding a PARP protein, or separate the 3' end of the sequence encoding the polypeptide tag from the 5' end of the sequence encoding the PARP protein. Sequences encoding the polypeptide tags are described in further detail below.

Polypeptide Tags

Polypeptide tags may be attached to a native protein sequence in order to aid in the purification of the protein, to label the protein for visualization in the cell, and/or to increase the thermodynamic stability and/or half-life of a protein. Nucleic acids encoding a polypeptide tag(s) may include one or more of the following sequences: a sequence encoding an epitope which may be recognized by a specific antibody recognizing the epitope (e.g., 1, 2, 3, 4 or 5 antigenic peptide sequences); a sequence encoding a protein that is bound with high affinity by a specific binding partner; one or more (e.g., 1, 2, 3, 4, or 5) sequence(s) encoding a peptide sequence that aids in purification (e.g., a His₆ tag); one or more (e.g., 1, 2, 3, 4, 5, 6, or 7) sequence(s) encoding a protease recognition sequence; and one or more (e.g., 1, 2, 3, or 4) sequences encoding a protein or a domain of a protein which increases the thermodynamic stability or half-life of the protein. The size of the nucleic acid sequence encoding the polypeptide tag may be between 1-50 nucleotides, 1-100 nucleotides, 1-200 nucleotides, 1-300 nucleotides, 1-400 nucleotides, 1-500 nucleotides, 200-500 nucleotides, 1-1,000 nucleotides, 1-5,000 nucleotides, 1-8,000 nucleotides, 1-10,000 nucleotides, or 1-20,000 nucleotides. Several polypeptide tags and sequences encoding polypeptide tags are known in the art. Non-limiting examples of sequences that may be incorporated in polypeptide tags are described below.

The nucleic acids encoding a polypeptide tag may contain sequences for one or more (e.g., 1, 2, 3, 4, or 5) epitopes or antigenic peptide sequences. Epitopes incorporated into polypeptide tags may be used to aid in the purification of a fusion protein, for e.g., by use of an antibody that specifically binds to the epitope. Examples of epitope sequences include, but are not limited to, a FLAG peptide (DYKDDDDK; SEQ ID NO: 30); a glutathione-S-transferase (GST) peptide; a KT3 peptide (KPPTPPPEPET; SEQ ID NO: 31); a hemagglutinin peptide (YPYDVPDYA; SEQ ID NO: 32); a calmodulin-binding peptide (*Methods in Molecular Biology: E. coli Gene Expression Protocols*, volume 205, Humana Press, 2003, pp. 79-97), a R-tag peptide (Jones et al., *Protein Expr. Purif.* 53:404-410, 2007), a V5 peptide, a c-myc peptide, and peptides derived from chitin-binding protein (CBP), CYD,

Strep II, HPC, and maltose binding protein (MBP), as described in Lichty et al. (*Protein Expr. Purif.* 41:98-105, 2005).

Nucleic acids encoding a polypeptide tag may contain sequences for one or more (e.g., 1, 2, 3, 4, or 5) proteins with specific binding partners. Desirably, the specific binding partner has a high affinity (e.g., $K_D < 150$ nM or $K_D < 250$ nM) to the peptide sequence in the polypeptide tag. Non-limiting examples of sequences that encode a protein with a high-affinity binding partner is biotin and the ZZ-domain of *S. aureus* protein A (e.g., a nucleic acid sequence with at least 80% identity to SEQ ID NO: 27). Additionally, the polypeptide tag may contain one or more peptide sequences that aid in the purification of the protein. Non-limiting examples of peptide sequences that aid in the purification of a protein include a His₆ tag, chitin-binding protein (CBP), maltose-binding protein (MBP), and glutathione-S-transferase (GST). For example, a protein containing a polypeptide tag containing a His₆ tag may be purified by passing a crude cellular lysate over a metal matrix (e.g., a Ni²⁺-Sephacrose resin).

A polypeptide tag may also contain a sequence encoding a protein that increases the thermodynamic stability, half-life, and/or solubility of a protein. Non-limiting examples of peptides that increase the solubility of a protein include thioredoxin and poly(NANP). Additional non-limiting examples of proteins that increase the thermodynamic stability or half-life of a protein include the Fc domain of an antibody and albumin. A polypeptide tag may also contain one or more (e.g., 1, 2, 3, or 4) sequences encoding a protein that allows for the visualization of the fusion protein in the cell (e.g., a polypeptide tag containing a sequence encoding a fluorescent protein, such as green fluorescence protein).

A polypeptide tag may also contain one or more (e.g., 1, 2, 3, 4, 5, 6, or 7) protease recognition sequences. A fusion protein may be treated with one or more (e.g., 1, 2, 3, or 4) specific proteases that cleave the fusion protein at the one or more specific protease recognition sequences at any step in the purification process (e.g., after being bound to a resin or solid surface) to remove the polypeptide tag(s) from the remainder of the fusion protein. Non-limiting examples of protease recognition sequences include TEV protease (Glu-X-X-Tyr-X-Gln-Ser; SEQ ID NO: 26), factor Xa (Ile-Glu/Asp-Gly-Arg), Ala-64 subtilisin (Gly-Ala-His-Arg), clostripain (Arg and Lys-Arg), collagenase (Pro-Val-Gly-Pro), enterokinase (Asp-Asp-Asp-Lys), renin (Pro-Phe-His-Leu-Leu), and α -thrombin (Leu-Val-Pro-Arg-Gly-Ser). When a polypeptide tag is present at the N-terminus of a fusion protein, a protease recognition sequence is preferably located at a position 3' to a peptide sequence encoding an epitope, a sequence encoding a protein that is bound with high affinity by a specific binding partner, or a sequence encoding a peptide sequence that aids in purification. When a polypeptide tag is present at the C-terminus of a fusion protein, a protease recognition sequence is preferably located at a position 5' to a peptide sequence encoding an epitope, a sequence encoding a protein that is bound with high affinity by a specific binding partner, and/or a sequence encoding a peptide sequence that aids in purification. A polypeptide tag may contain one or more (e.g., 1, 2, 3, 4, 5, 6, or 7) of the same or different protease recognition sequences in tandem (i.e., without intervening amino acids) or with one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) intervening amino acids between each protease recognition sequence. Methods for the treatment of a fusion protein containing a protease recognition sequence in the polypeptide tag with one or more protease(s) are known in the art.

Expression Vectors

A number of expression vectors for the expression of a nucleic acid encoding one or more nucleic acids encoding a PARP, PARG, and/or ARH3 fusion protein of the invention are known in the art. Different examples of expression vectors are available for expression of the PARP, PARG, and/or ARH3 fusion proteins in mammalian cells, insect cells, yeast cells, and bacterial cells. For example, the pEGFP-C1 mammalian vector (Invitrogen) contains a CMV promoter sequence, a nucleic acid sequence encoding green fluorescence protein, a multiple cloning site for insertion of nucleic acid sequence encoding a PARP, PARG, or ARH3 nucleic acid (e.g., a sequence with 80% to one of SEQ ID NOS: 1-24, 41, and 42). Additional non-limiting examples of publicly-available mammalian expression vectors include constitutive expression vectors Gateway® pDEST™ 26, pDEST™ 27, pDEST™ 40, and pDEST™ 47 (Invitrogen); adenoviral expression vectors (e.g., pAd/CM/V5-Dest Gateway® Vector Kit (Invitrogen); episomal expression vectors pCEP4 and pEBNA DEST (Invitrogen); lentiviral expression vectors (e.g., ViraPower™ Bsd; Invitrogen); and regulated expression vectors Gateway® pT-Rex™-DEST 30 and pT-Rex™-DEST 31 (Invitrogen). Non-limiting examples of bacterial expression vectors include Gateway® pDEST™ 14; Gateway® pDEST™ 15; Gateway® pDEST™ 17; Gateway® pDEST™ 24; Gateway® pET-DEST42; pEM7/Bsd; pEM7/Zeo; pRSET A, B, & C; pRSET-BFP; pRSET-CFP; pRSET-EmGFP; pTrcHis A, B, & C; and pTrcHis2 A, B, & C vectors (Invitrogen). Non-limiting examples yeast expression vectors include pAO815; pGAPZ A, B, & C; pPIC3.5K; pPIC9K; pTEF1/Bsd; pTEF1/Zeo; pYC2/CT; pYES2; pYES2/CT; and pYES3/CT (Invitrogen). Non-limiting examples of insect and baculovirus expression vectors include Gateway® pDEST™ 10; Gateway® pDEST™ 20; Gateway® pDEST™ 8; Gateway® pMT-DEST™ 48; pAC5.1/V5-His A, B, & C; pFast-Bac Dual; and pIB/V5-His-DEST (Invitrogen).

The expression vectors used to express a fusion protein may include one or more (e.g., 1, 2, 3, 4, or 5) constitutive promoter sequences and/or one or more (e.g., 1, 2, 3, 4, or 5) inducible promoter sequences. Non-limiting examples of constitutive promoter sequences include bacterial promoters (e.g., *E. coli* σ^{70} , σ^S , σ^{32} , or σ^{54} promoters; *B. subtilis* σ^A or σ^B promoters; T7 RNA polymerase-based promoters; and a bacteriophage SP6 promoter), yeast promoters (e.g., pCyc, pAdh, pSte5, ADH1, cyc100, cyc70, cyc43, cyc28, cyc16, pPGK1, pCYC, GPD (TDH3), and CLB1 promoters), and mammalian promoters (e.g., cytomegalovirus immediate early gene-based promoters, SV40 early promoter, and Rous sarcoma virus promoter). Non-limiting examples of inducible promoter sequences include alcohol dehydrogenase I gene promoters, tetracycline-responsive promoter systems, glucocorticoid receptor promoters, estrogen receptor promoter, ecdysone receptor promoters, metallothionein-based promoters, and T7-polymerase based promoters. Several different mammalian expression vectors available that allow for the inducible expression of a nucleic acid sequence (e.g., a PARP fusion protein) are publicly available including pTet-On-Advanced (Clontech), pERV3 (Stratagene), pNEBR-R1 (New England BioLabs), and pCMV5-CymR (Qbiogene).

PARP, PARG, and ARH3 Proteins

The above-described methods for the generation of PARP, PARG, or ARH3 fusion proteins may be modified to generate PARP, PARG, or ARH3 proteins. In these methods, the expression vectors that contain a nucleic acid sequence encoding a PARP, PARG, or ARH3 fusion protein may be modified to remove the nucleic acid sequences encoding the polypeptide tag. The modified vector may then be introduced

into a cell to generate a transgenic cell for the expression of the full-length or wild-type PARP, PARG, or ARH3 protein. The produced PARP, PARG, or ARH3 protein may contain one or more post-translational modifications, including phosphorylation and poly-ADP-riboylation. The post-translational modifications may be introduced using recombinant enzymes in vitro or may be the result of processing within the transgenic cell.

Methods for the expression and purification of one or more PARP, PARG, and ARH3 proteins are the same as those employed for the corresponding PARP, PARG, and ARH3 fusion proteins, with the exception that affinity purification using antibodies and molecules that specifically recognize the polypeptide tag will not be employed.

Transgenic Cells and Mammals

One or more nucleic acids encoding a PARP, PARG, and/or ARH3 protein, and/or PARP, PARG, and/or ARH3 fusion protein may be introduced into a transgenic cell using methods known in the art, including, but not limited to electroporation, microinjection, lipid-mediated transfection (e.g., liposomal delivery systems), calcium phosphate-mediated transfection, DEAE dextran-mediated transfection, DNA transfection by biolistics, DNA transfection mediated by polybrene, and virus-mediated transduction.

The one or more nucleic acids encoding a PARP, PARG, and/or ARH3 protein, and/or PARP, PARG, and/or ARH3 fusion protein may be introduced into any type of cell, including, but not limited to, a mammalian cell (e.g., a human, mouse, rat, monkey, or rabbit cell), a yeast cell, a bacterial cell, or an insect cell. A mammalian cell that expresses one or more nucleic acids encoding a PARP, PARG, and/or ARH3 protein, and/or PARP, PARG, and/or ARH3 fusion protein may include a fibroblast, an epithelial cell, an endothelial cell, a smooth muscle cell, a hepatocyte, a kidney cell, and a lymphocyte. Additional examples of suitable mammalian cell lines include COS-7 monkey kidney cells, CV-1, L cells, C127 cells, 3T3 cells, Chinese hamster ovary (CHO) cells, human embryonic kidney (HEK) cells, HeLa cells (e.g., HeLa S3 or HeLa Kyoto cells), 293 cells, 293T cells, and BHK cell lines. One or more nucleic acids may also be expressed in a cell (e.g., a mammalian cell, a bacterial cell, or a yeast cell) that has been engineered to express one or more (e.g., 1, 2, 3, or 4) chaperone proteins, one or more (e.g., 1, 2, 3, or 4) enzymes that promote the post-translational modification of proteins, and/or contain one or more (e.g., 1, 2, 3, or 4) mutations in the nucleic acids encoding one or more (e.g., 1, 2, 3, or 4) proteins that have a negative effect on the expression of a transgenic protein (e.g., a PARP fusion protein), such as a specific RNase or protease. An example of a bacterial cell that has been engineered to contain a mutation in a RNase is BL21 Star™ (Invitrogen). A variety of cells are commercially available for the expression of one or more recombinant proteins (e.g., one or more PARP, PARG, and/or ARH3 fusion proteins), including, but not limited to, bacterial competent cells (e.g., BL21-AI™ One Shot®, One Shot®-BL21(DE3), and One Shot®-BL21(DE3) pLysE, One Shot® BL21(DE3) pLysS (Invitrogen); and mammalian competent cells (e.g., Espresso Competent HeLa S3 Cells, Espresso Competent CHO-K1 cells, and Espresso Competent HEK 293 cells (Neuromics), MaxPAK Competent HeLa S3 cells, MaxPAK Competent CHO-K1 cells, and MaxPAK Competent HEK 293 cells (Genlantis)).

A transgenic cell that contains one or more nucleic acids encoding a PARP, PARG, and/or ARH3 protein, and/or PARP, PARG, and/or ARH3 fusion protein may a stable cell line (e.g., a cell that has integrated the one or more nucleic acids encoding a PARP fusion protein into one or more of its

chromosomes). Alternatively, a transgenic cell may contain the one or more nucleic acids encoding a PARP, PARG, and/or ARH3 protein, and/or PARP, PARG, and/or ARH3 fusion protein in a plasmid or on an artificial chromosome, which replicates independently of the chromosomes of the cell.

A transgenic mammal may also be produced from a transgenic cell containing one or more nucleic acids encoding a PARP, PARG, and/or ARH3 protein, and/or a PARP, PARG, and/or ARH3 fusion protein. A transgenic animal may be a mouse, a rat, a bovine, an ovine, a caprine, a porcine, a horse, a rabbit, or a monkey. The nucleic acid encoding one or more PARP, PARG, and/or ARH3 proteins, and/or PARP, PARG, and/or ARH3 fusion proteins may contain a tissue-specific promoter that allows the expression of one or more transgenic proteins into a biological fluid of the mammal (e.g., into the milk or serum of the transgenic mammal). For example, a protein may be engineered for expression in the milk of a mammal by placing the sequence encoding the protein downstream of the casein promoter (U.S. Pat. No. 4,873,316). A PARP, PARG, and/or ARH3 protein, and/or PARP, PARG, and/or ARH3 fusion protein produced in a biological fluid of a transgenic mammal may be purified as described below.

Methods for the production of a transgenic mammal from a transgenic cell are known in the art and include, without limitation, methods that require the transfer of a nucleus from a transgenic cell to an enucleated oocyte and/or the microinjection of one or more nucleic acids (e.g., a plasmid or an artificial chromosome) encoding one or more PARP, PARG, and/or ARH3 proteins, and/or PARP, PARG, and/or ARH3 fusion proteins into an oocyte. Such genetically manipulated oocytes may then be transferred into a recipient female host to produce a transgenic mammal.

Cell Lysates

Cell lysates may be prepared from the transgenic cells containing a nucleic acid encoding one or more PARP, PARG, and/or ARH3 proteins, and/or PARP, PARG, and/or ARH3 fusion proteins of the invention. Cell lysates may be prepared by any methods known in the art, including both physical disruption methods and chemical disruption methods. Physical disruption methods include, but are not limited to sonication, homogenization, and rapid freeze/thaw lysis. Chemical disruption methods include, but are not limited to, the use of lysis buffers (e.g., buffers containing a detergent such as Triton-X-100 and NP-40). Following lysis of the cell membrane using chemical and/or physical disruption methods, the lysate may optionally be centrifuged to remove cellular debris and/or partially purified by one or more (e.g., 1, 2, 3, 4, 5, 6, or 7) of the following steps: salt gradient precipitation (e.g., ammonium sulfate precipitation), size exclusion chromatography or dialysis, and column chromatography (e.g., affinity chromatography, size exclusion chromatography, anion exchange chromatography, and cation exchange chromatography). The cell lysate may also be treated with one or more (e.g., 1, 2, or 3) of a DNase, RNase, or lipase prior to further use. One or more (e.g., 1, 2, 3, 4, or 5) protease inhibitors may also be added to the cell lysate prior to use.

PARP, PARG, and ARH Protein and Fusion Protein Purification

One or more PARP, PARG, and/or ARH3 proteins, and/or PARP, PARG, and/or ARH3 fusion proteins may be fully or partially purified (e.g., at least 60%, 70%, 80%, 85%, 90%, 95%, and 99% pure from other proteins in the cell) from cell lysates, a biological medium (e.g., cell culture medium from a transgenic cell), or a biological fluid (e.g., blood, serum, or milk) from transgenic mammal expressing one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or

20 or more) nucleic acids encoding a PARP, PARG, and/or ARH3 protein, and/or a PARP, PARG, and/or ARH3 fusion protein of the invention. Alternatively, one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 or more) PARP, PARG, and/or ARH3 proteins, and/or PARP, PARG, and/or ARH3 fusion proteins may be fully or partially purified from the extracellular medium of a transgenic cell expressing one or more nucleic acids encoding a PARP, PARG, and/or ARH3 protein, and/or a PARP, PARG, and/or ARH3 fusion protein of the invention. In each example, a cell lysate, biological fluid (e.g., milk or serum), or extracellular medium containing one or more PARP, PARG, and/or ARH3 proteins, and/or PARP, PARG, and/or ARH3 fusion proteins is collected.

Methods for the purification of a recombinant protein from a cell lysate, biological fluid, or extracellular medium are known in the art. For example, in instances where the PARP, PARG, and/or ARH3 fusion protein contains an epitope, an antibody specific for the epitope (e.g., anti-GFP antibodies, anti-FLAG antibodies, anti-GST antibodies, anti-hemagglutinin antibodies, anti-c-myc antibodies, and anti-V5 antibodies) may be used to purify one or more PARP, PARG, and/or ARH3 fusion protein(s). In another example, a PARP, PARG, and/or ARH3 fusion protein may contain a polypeptide tag containing a sequence that aids in affinity purification of the protein (e.g., a His₆ tag, a calmodulin-binding protein tag, a glutathione S-transferase protein tag, a strep II tag, a HPC tag, a maltose-binding protein tag). In each example, a solid surface, resin, or bead (e.g., magnetic bead) may be covalently attached to a protein or molecule specifically bound by the protein sequence located in the polypeptide tag. In such instances, contacting the one or more PARP, PARG, and/or ARH3 fusion protein(s) with the solid surface, resin, or bead will cause the selective binding of the one or more PARP, PARG, and/or ARH3 fusion protein(s) with the solid surface, resin, or bead. The remaining non-bound proteins will not bind and may be washed away using an appropriate buffer. Specific methods for the affinity purification of proteins are known in the art.

One or more PARP, PARG, and/or ARH3 fusion proteins may also be purified from a cell lysate, biological sample, or a extracellular medium by a purification protocol including, but limited to: salt precipitation (e.g., ammonium sulfate precipitation), pH precipitation, precipitation using organic solvents, high performance liquid chromatography (HPLC), column chromatography, ion exchange chromatography (e.g., cation exchange chromatography and anion exchange chromatography), immobilized metal affinity chromatography, gel filtration, or size exclusion chromatography or dialysis. One or more (e.g., 1, 2, 3, 4, 5, 6, or 7) of these steps may also be used in combination with an affinity purification step as described above.

The one or more purified PARP, PARG, and/or ARH3 proteins, and/or PARP, PARG, and/or ARH3 fusion proteins may be dialyzed to exchange the buffer or concentrated prior to use in one or more of the assays described herein (e.g., PARP, PARG, and/or ARH3 activity assays or assays for the identification of a specific PARP activator or inhibitor). The one or more purified PARP, PARG, and/or ARH3 proteins, and/or PARP, PARG, and/or ARH3 fusion proteins may be stored at -70° C. in the presence or absence of one or more (e.g., 1, 2, 3, 4, or 5) stabilizing proteins including, but not limited to, albumin.

PARP Protein and PARP Fusion Protein Assays

The biological activity of the one or more PARP proteins and PARP fusion proteins of the invention include, but are not limited to, one or more (e.g., 1, 2, 3, 4, or 5) of the ability to

covalently attach an ADP-ribose molecule to one or more (e.g., 1, 2, 3, 4, or 5) substrate(s) (e.g., a protein, a RNA molecule, a DNA molecule, or a lipid), the ability to covalently attach an ADP-ribose molecule to a ADP-ribose residue covalently attached to a substrate, the ability to add a branched ADP-ribose molecule to a pre-existing poly-ADP-ribose, the ability to localize to the cell nucleus, the ability to localize to stress granules, the ability to catalyze the formation or nucleation of stress granules, the ability to catalyze the disassembly of stress granules, the ability to promote cell division and mitosis, or the ability to inhibit RNAi activity in the cell. Specific PARP proteins have a different subset of biological activities: PARP1, PARP2, PARP5A, PARP5B, PARP7, PARP8, PARP14, and PARP16 have the ability to localize to the nucleus and/or the ability to promote cell division and mitosis; PARP5A, PARP12, PARP13.1, PARP13.2, and PARP15 have the ability to localize to stress granules and the ability to promote or nucleate stress granule formation; PARP11 has the ability to localize to stress granules and the ability to promote disassembly of stress granules or prevent the formation of stress granules; and PARP13.1 has the ability to decrease the activity of RNAi and the ability to add one or more ADP-ribose molecules to Argonaut.

Assays to measure the ability of one or more PARP proteins and PARP fusion protein(s) to covalently attach an ADP-ribose to one or more (e.g., 1, 2, 3, 4, or 5) substrate(s) (e.g., a protein, a RNA, a DNA, or a lipid) involve the incubation of one or more PARP fusion protein(s) with the one or more substrate(s) in the presence of a labeled NAD⁺ molecule (e.g., radiolabeled, fluorescently-labeled, and colorimetrically-labeled NAD⁺). A radiolabeled NAD⁺ substrate may contain one or more radioisotopes including, but not limited to, ¹⁴C (e.g., ¹⁴C-adenine), ³²P, and ³H. Additional NAD⁺ substrates include fluorescently-labeled NAD⁺ (Putt et al., *Anal. Biochem.* 78:326, 2004), colorimetrically-labeled NAD⁺ (Nottbohn et al., *Agnew. Chem. Int. Ed.* 46:2066-2069, 2007), and biotinylated NAD⁺ (6-biotin-17-NAD; R & D Systems). Following incubation of the one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 or more) PARP proteins and/or the one or more PARP fusion proteins with the labeled NAD⁺ and one or more (e.g., 1, 2, 3, 4, or 5) substrate molecules, the specific labeling of the substrate(s) with one or more labeled ADP-ribose molecules is determined by measuring the amount of the label associated with the NAD⁺ that is covalently bound to the one or more substrate molecules. An increase (e.g., at least 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%) in the amount of the label associated with the NAD⁺ that is covalently bound to the one or more substrate(s) indicates PARP protein or PARP fusion protein activity.

In another example of a PARP assay, the auto-modification of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 or more) PARP proteins or PARP fusion protein(s) is measured by incubating the one or more PARP proteins and/or PARP fusion proteins of the invention with a labeled NAD⁺ substrate and subsequently, measuring the amount of the label associated with the NAD⁺ covalently bound to the one or more PARP fusion proteins. An increase in the amount of the label associated with the NAD⁺ covalently bound to the one or more PARP fusion proteins indicates PARP fusion protein auto-modification.

In an alternative assay, one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 or more) PARP proteins and/or PARP fusion proteins may be incubated with one or more (e.g., 1, 2, 3, 4, or 5) substrates and a non-labeled NAD⁺. The poly-ADP-ribosylation of the one or more substrates may be measured by contacting the one or more sub-

strates with a poly-ADP-ribose antibody. For example, a sample of substrate proteins may be electrophoresed and immunoblotted with an anti-poly-ADP-ribose antibody. An increased number of proteins or an increased level of detection using an anti-poly-ADP ribose antibody indicates an increase in the activity of the one or more PARP fusion proteins.

Assays to measure the ability of a PARP protein and/or PARP fusion protein to localize to a specific cellular structure or organelle using immunofluorescence microscopy are known in the art. For example, antibodies specific for one or more PARP proteins and/or fusion proteins, and antibodies specific for one or more proteins or molecules specific to a cellular structure or organelle (e.g., cytoskeleton, mitochondria, trans-Golgi network, endoplasmic reticulum, early endosome, centrosome, GW bodies, nuclear envelope, lysosome, peroxisomes, histones, Cajal bodies, nucleus, and mitochondria) may be used to perform immunofluorescent microscopy. Localization of one or more PARP proteins and/or PARP fusion proteins may be measured in high-throughput experiments by co-localization of one or more PARP proteins and/or fusion proteins with one or more proteins specific for a cellular structure or organelle (e.g., proteins listed in FIG. 10). Localization of one or more PARP proteins and/or PARP fusion proteins in the nucleus may also be demonstrated by co-localization of a dye that stains DNA and an antibody that specifically binds the one or more PARP proteins and/or PARP fusion proteins (e.g., co-localization of an antibody specific for one or more PARP proteins and/or PARP fusion proteins, and 4',6-diamidino-2-phenylindole (DAPI)).

Localization of one or more PARP proteins and/or PARP fusion proteins to a specific cell structure or organelle may occur only during one or more (e.g., 1, 2, 3, 4, 5, or 6) specific stages of the cell cycle, including, but not limited to, G2-M, prophase, prometaphase (P-M), metaphase, anaphase, cytokinesis, G₀, and G₁ stages. For the purposes described herein, a PARP protein and/or PARP fusion protein is deemed to have the ability to localize to a specific cellular structure or organelle if it localizes to the specific cellular structure or organelle in at least one stage (e.g., mitosis or cytokinesis) of the cell cycle.

The ability of a PARP protein and/or fusion protein to promote stress granule assembly or to inhibit stress granule assembly may be measured using fluorescence microscopy. In such a method, cells are treated with one or more PARP inhibitors, one or more PARP activators, or a nucleic acid encoding one or more PARP proteins and/or PARP fusion proteins, and are subsequently fixed and immunostained with antibodies specific for one or more stress granule protein (e.g., one or more of eIF3, eIF1A, eIF2 α , eIF3 η , eIF4A1, eIF4e, and G3BP). An increase in the number of foci containing one or more stress granule proteins (e.g., intense immunostaining in distinct cellular structures) indicates an increase in the formation of stress granules. A decrease in the number of foci containing one or more stress granule proteins, likewise, indicates a decrease in the formation of stress granules. In such assays, stress granule formation may be induced by exposure to stress conditions, for example, by treatment with sodium arsenite and pateamine A.

The ability of one or more PARP proteins and/or PARP fusion proteins to promote cell division and mitosis may be measured using any method known in the art. For example, cell proliferation assays including, but not limited to, standard cell counting assays, BrdU labeling, and quantitative assays for DNA synthesis such as ³H-thymidine incorporation may be used to measure the ability of one or more PARP proteins and/or PARP fusion proteins to promote cell division

and mitosis. Likewise, inhibition of one or more PARP proteins and/or PARP fusion proteins with the ability to promote cell division and mitosis may result in cell death. Several assays to measure cell death are known in the art, including, but not limited to Hoechst 33342 staining of chromatin, propidium iodide staining, annexin V staining of phosphoserine, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) staining.

Assays for measuring RNAi activity in a cell are available in the art. For example, psiCHECK™-1, psiCHECK™-2, and pGL4.72[hRlucCP]™ vector assay systems provide methods for the measurement of RNAi activity in a cell. In these assays systems, luciferase is used a primary reporter gene and a target sequence (i.e., the target of one or more RNAi molecules) is cloned a multiple cloning region located downstream of the luciferase translational stop codon. Initiation of the RNAi process towards the target gene results in the cleavage and subsequent degradation of the fusion mRNA encoded by the vectors (i.e., upon treatment of the transfected cell with a vector-target RNAi molecule). Measurement of decreased luciferase activity in the transfected cells following treatment with the vector-target RNAi indicates the activity of RNAi in the cell. For example, in experiments using the psiCHECK assay system, a cell transfected with the psiCHECK vector is treated with the vector-target RNAi and with an activator or inhibitor of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARP proteins and/or PARP fusion proteins (e.g., 1, 2, 3, 4, or 5 RNAi molecules targeting a specific PARP). Transfected cells treated with the vector-target RNAi and with a PARP inhibitor or activator that demonstrate increased luciferase activity relative to a transfected cell treated with the vector-target RNAi alone indicate that the specific targeted PARP activates or inhibits RNAi activity in the cell, respectively. Cells treated with a PARP inhibitor or activator that demonstrate decreased luciferase activity relative to a cell treated with vector-target RNAi alone indicate that the specific targeted PARP inhibits or activates RNAi activity in the cell, respectively.

Any of the above-referenced PARP protein and/or PARP fusion protein activity assays may be performed to determine the activity of PARP protein or PARP fusion protein sequence encoded by a nucleic acid having at least 80% sequence identity to one of SEQ ID NOS: 1-24. The domain structure of several PARP proteins are shown in FIG. 3. Preferred mutations in the wild-type sequences of PARP proteins (e.g., SEQ ID NOS: 1-24) do not introduce amino acid changes in the catalytic domain in FIG. 3. In addition, the biological activity of a PARP protein and/or PARP fusion protein containing a sequence having at least 80% sequence identity to one of SEQ ID NOS: 1-24 may be assessed using any of the above-described cellular or in vitro assays.

PARG and ARH3 Protein and Fusion Protein Assays

The activity of one or more PARG protein, ARH3 protein, PARG fusion protein, and/or ARH3 fusion protein may be determined using assays known in the art. PARG and ARH3 proteins herein have been demonstrated to decrease or prevent the formation of stress granules. Assays for the measurement of stress granule formation and disassembly are described herein.

Additional assays for PARG and ARH3 proteins (and fusion proteins) include the hydrolysis of poly-ADP-ribose. Labeled poly-ADP-ribose (e.g., ³²P, ¹⁴C, or biotinylated ADP) may be used as a substrate for the measurement of the hydrolysis and release of ADP-ribose from a labeled and/or attached poly-ADP-ribose polymer.

Additional assays for PARG and ARH3 proteins involve the use of antibodies specific for poly-ADP-ribose. For

example, cells may be transfected with a nucleic acid that overexpresses a PARG protein, ARH3 protein, PARG fusion protein, and/or ARH3 fusion protein and cells untreated or treated with a stress condition (e.g., sodium arsenite). Cells that contain an active form of a PARG protein, ARH3 protein, PARG fusion protein, and/or ARH3 fusion protein show decreased staining for poly-ADP-ribose than cells transfected with a control form or inactive form of these proteins (e.g., a form lacking its catalytic domain or a form containing an inactivating mutation).

These activity assays will also aid in the identification of which amino acids may be mutated to generate nucleic acids having at least 80% (e.g., at least 85%, 90%, 95%, 99%, or 100% identity) to PARG (SEQ ID NO: 42) or ARH3 (SEQ ID NO: 41). Preferred sites of mutation lie outside the catalytic domains of PARG and/or ARH3.

PARP-Specific Antibodies

Antibodies specific to the one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 or more) PARP proteins and/or PARP fusion proteins of the invention can be generated using standard methods, such as those described herein. Antibodies specific for one or more PARP fusion proteins, PARP proteins, or fragments of PARP proteins and/or PARP fusion proteins may be used in quantitative assays to measure to amount of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 or more) PARP proteins and/or PARP fusion proteins present in a cell, cell lysate, biological sample, or extracellular medium. Antibodies specific to the one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 or more) PARP proteins and/or PARP fusion proteins of the invention may also be used to identify specific binding partners or potential inhibitors or activators of the one or more PARP proteins and/or PARP fusion proteins.

For the preparation of polyclonal antibodies reactive with one or more PARP proteins and/or PARP fusion proteins, one or more PARP protein(s), PARP fusion protein(s), fragments of PARP protein(s), or fragments of PARP fusion protein(s) can be purified from natural sources (e.g., cultures of cells expressing one or more PARP proteins) or synthesized in, e.g., mammalian, insect, or bacterial cells by expression of corresponding DNA sequences contained in a suitable cloning vehicle (e.g., the nucleic acids encoding PARP proteins and PARP fusion proteins described herein). Fusion proteins are commonly used as a source of antigen for producing antibodies. The antigenic proteins can be optionally purified, and then coupled to a carrier protein, mixed with Freund's adjuvant to enhance stimulation of the antigenic response in an inoculated animal, and injected into rabbits, mice, or other laboratory animals. Primary immunizations are carried out with Freund's complete adjuvant and subsequent immunizations performed with Freund's incomplete adjuvant. Following booster injections at bi-weekly intervals, the inoculated animals are then bled and the sera isolated. The sera is used directly or is purified prior to use by various methods, including affinity chromatography employing reagents such as Protein A-Sepharose, antigen-Sepharose, and anti-horse-Ig-Sepharose. Antibody titers can be monitored by Western blot and immunoprecipitation analyses using one or more PARP proteins, PARP fusion proteins, and/or fragments of PARP fusion proteins or PARP proteins. Immune sera can be affinity purified using one or more PARP proteins, PARP fusion proteins, and/or fragments of PARP fusion proteins or PARP proteins coupled to beads. Antiserum specificity can be determined using a panel of proteins, such as one or more PARP proteins, PARP fusion proteins, and/or fragments of PARP fusion proteins or PARP proteins.

Alternatively, monoclonal antibodies are produced by removing the spleen from the inoculated animal, homogenizing the spleen tissue, and suspending the spleen cells suspended in phosphate buffered saline (PBS). The spleen cells serve as a source of lymphocytes, some of which produce antibody of the appropriate specificity. These cells are then fused with permanently growing myeloma partner cells, and the products of the fusion plated into a number of tissue culture wells in the presence of selective agents, such as hypoxanthine, aminopterin, and thymidine (Mocikat, *J. Immunol. Methods* 225:185-189, 1999; Jonak et al., *Hum. Antibodies Hybridomas* 3:177-185, 1992; Srikumaran et al., *Science* 220:522, 1983). The wells can then be screened by ELISA to identify those containing cells making antibody capable of binding to one or more PARP proteins, PARP fusion proteins, fragments of PARP proteins, and/or fragments of PARP fusion proteins, or mutants thereof. These cells can then be re-plated and, after a period of growth, the wells containing these cells can be screened again to identify antibody-producing cells. Several cloning procedures can be carried out until over 90% of the wells contain single clones that are positive for specific antibody production. From this procedure, a stable cell line of clones that produce the antibody are established. The monoclonal antibody can then be purified by affinity chromatography using Protein A Sepharose and ion-exchange chromatography, as well as variations and combinations of these techniques. Once produced, monoclonal antibodies are also tested for specific PARP protein and/or PARP fusion protein recognition by ELISA, Western blot, and/or immunoprecipitation analysis (see, e.g., Kohler et al., *Nature* 256:495, 1975; Kohler et al., *Eur. J. Immunol.* 6:511, 1976; Kohler et al., *Eur. J. Immunol.* 6:292, 1976; Hammerling et al., In *Monoclonal Antibodies and T Cell Hybridomas*, Elsevier, New York, N.Y., 1981).

As an alternate or adjunct immunogen to a PARP protein and/or PARP fusion protein, peptides corresponding to relatively unique regions of a PARP protein or PARP fusion protein can be generated and coupled to keyhole limpet hemocyanin (KLH) through an introduced C-terminal lysine. Antiserum to each of these peptides can be similarly affinity-purified on peptides conjugated to BSA, and specificity tested by ELISA and Western blotting using peptide conjugates, and by Western blotting and immunoprecipitation using a PARP protein, PARP fusion protein, and/or fragment of a PARP protein or PARP fusion protein.

Antibodies of the invention are desirably produced using PARP protein and/or PARP fusion protein amino acid sequences that do not reside within highly conserved regions, and that appear likely to be antigenic, as evaluated by criteria such as those provided by the Peptide Structure Program (Genetics Computer Group Sequence Analysis Package, Program Manual for the GCG Package, Version 7, 1991) using the algorithm of Jameson et al., *CABIOS* 4:181, 1988. These fragments can be generated by standard techniques, e.g., by PCR, and cloned into any appropriate expression vector. For example, GST fusion proteins can be expressed in *E. coli* and purified using a glutathione-agarose affinity matrix. To minimize the potential for obtaining antisera that is non-specific or exhibits low-affinity binding to one or more PARP proteins, PARP fusion proteins, and/or fragments of PARP proteins or PARP fusion proteins, two or three PARP fusion proteins may be generated for each fragment injected into a separate animal. Antisera are raised by injections in series, preferably including at least three booster injections.

In addition to intact monoclonal and polyclonal anti-PARP protein or anti-PARP fusion protein antibodies, various genetically engineered antibodies and antibody fragments

(e.g., F(ab')₂, Fab', Fab, Fv, and sFv fragments) can be produced using standard methods. Truncated versions of monoclonal antibodies, for example, can be produced by recombinant methods in which plasmids are generated that express the desired monoclonal antibody fragment(s) in a suitable host. Ladner (U.S. Pat. Nos. 4,946,778 and 4,704,692) describes methods for preparing single polypeptide chain antibodies. Ward et al., *Nature* 341:544-546, 1989, describes the preparation of heavy chain variable domain which have high antigen-binding affinities. McCafferty et al. (*Nature* 348:552-554, 1990) show that complete antibody V domains can be displayed on the surface of fd bacteriophage, that the phage bind specifically to antigen, and that rare phage (one in a million) can be isolated after affinity chromatography. Boss et al. (U.S. Pat. No. 4,816,397) describes various methods for producing immunoglobulins, and immunologically functional fragments thereof, that include at least the variable domains of the heavy and light chains in a single host cell. Cabilly et al. (U.S. Pat. No. 4,816,567) describes methods for preparing chimeric antibodies. In addition, the antibodies can be coupled to compounds, such as toxins or radiolabels.

Methods for Identification of Specific PARP Inhibitors or Activators

The PARP proteins and/or PARP fusion proteins of the invention may be used to identify one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 or more) specific PARP activators or inhibitors. In the provided assays, one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 or more) PARP proteins and/or PARP fusion proteins are contacted with an agent (e.g., a test agent), a labeled NAD⁺ (e.g., a colorimetrically-labeled, fluorescently-labeled, biotinylated-, or radioisotope-labeled NAD⁺), and one or more substrates, and measuring the amount of labeled ADP-ribose covalently attached to the one or more substrates. In one example, one or more PARP protein and/or PARP fusion proteins of the invention are incubated with a labeled NAD⁺ substrate and the amount of label associated with the NAD⁺ that is covalently attached to the one or more PARP proteins and/or PARP fusion proteins is measured (e.g., auto-modulation activity assay). In this example, an agent that is a specific PARP inhibitor mediates a decrease (e.g., at least a 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 80%, 90%, 95%, or even 100% decrease) in the amount of labeled ADP-ribose covalently attached to one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) PARP proteins and/or fusion proteins, wherein the label on the PARP-fusion proteins is the same as the label of the NAD⁺. In a method for identifying an agent that is a specific PARP activator, the agent mediates an increase (e.g., at least a 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 80%, 90%, 95%, or even 100% increase) in the amount of labeled ADP-ribose covalently attached to one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20) PARP proteins and/or PARP fusion proteins.

The one or more PARP proteins and/or PARP fusion proteins utilized in each assay may be purified, partially purified (e.g., at least 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, or 95% pure) or may be present in a cell lysate (e.g., a bacterial cell lysate, a yeast cell lysate, or a mammalian cell lysate), in a biological fluid from a transgenic animal (e.g., milk or serum), or an extracellular medium. The one or more PARP proteins and/or PARP fusion proteins utilized in the assay may be bound to substrate, such as, but not limited to, a solid surface (e.g., a multi-well plate), a resin, or a bead (e.g., a magnetic bead).

In additional examples of the assays, the one or more PARP proteins and/or PARP fusion proteins may be bound to a solid surface, resin, or bead (e.g., a magnetic bead) and subsequently treated with one or more protease(s) (e.g., a TEV protease) prior to contacting the one or more PARP proteins and/or PARP fusion proteins with the labeled NAD⁺.

In preferred assays, an activator or inhibitor increases or decreases the amount of labeled ADP-ribose covalently attached to a specific PARP protein, PARP fusion protein, and/or subset of PARP proteins or fusion proteins while having no or little (e.g., less than 50%, 40%, 30%, 25%, 20%, 15%, 10%, or 5% change (e.g., increase or decrease)) affect on the amount of labeled ADP-ribose covalently attached to other PARP proteins and/or PARP fusion proteins, is identified as a PARP activator or inhibitor, respectively. For example, the assay desirably identifies an agent that specifically inhibits the amount of labeled ADP-ribose covalently attached to one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARP5A proteins or fusion proteins, PARP12 proteins and/or fusion proteins, PARP13.1 proteins and/or fusion proteins, PARP13.2 proteins and/or fusion proteins, and PARP15 proteins and/or fusion proteins. Another assay desirably identifies an agent that specifically increases the amount of labeled ADP-ribose covalently attached to one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARP5A proteins and/or fusion proteins, PARP12 proteins and/or fusion proteins, PARP13.1 proteins and/or fusion proteins, PARP 13.2 proteins and/or fusion proteins, and PARP15 proteins and/or fusion proteins. Another example of the assay desirably identifies an activator or inhibitor that specifically increases or decreases, respectively, the amount of labeled ADP-ribose covalently bound to one or more (e.g., 1, 2, 3, 4, 5, or 6) PARP11 proteins and/or fusion proteins. Another example of the assay desirably identifies an agent that specifically decreases the amount of labeled ADP-ribose covalently attached to one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARP1 proteins and/or fusion proteins, PARP2 proteins and/or fusion proteins, PARP5A proteins and/or fusion proteins, PARP5B proteins and/or fusion proteins, PARP7 proteins and/or fusion proteins, PARP8 proteins and/or fusion proteins, PARP14 proteins and/or fusion proteins, and PARP16 proteins and/or fusion proteins. Another example of the assay desirably identifies an agent that specifically increases the amount of labeled ADP-ribose covalently attached to one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARP1 proteins and/or fusion proteins, PARP2 proteins and/or fusion proteins, PARP5A proteins and/or fusion proteins, PARP5B proteins and/or fusion proteins, PARP7 proteins and/or fusion proteins, PARP8 proteins and/or fusion proteins, PARP14 proteins and/or fusion proteins, and PARP16 proteins and/or fusion proteins. In another desirable embodiment of the assay, the assay identifies an agent that specifically increases or decreases the amount of labeled ADP-ribose covalently attached to one or more (e.g., 1, 2, 3, 4, 5, or 6) different PARP13.1 proteins and/or fusion proteins.

A variety of different agents may be tested in the above-described assays provided by the invention. For example, a tested agent may be a derived from or present in a crude lysate (e.g., a lysate from a mammalian cell or plant extract) or be derived from a commercially available chemical libraries. Large libraries of natural product or synthetic (or semi-synthetic) extracts or chemical libraries are commercially available and known in the art. The screening methods of the present invention are appropriate and useful for testing agents from a variety of sources for activity as a specific PARP activator or inhibitor. The initial screens may be performed using a diverse library of agents, but the method is suitable for a variety of other compounds and compound libraries. Such

compound libraries can also be combinatorial libraries. In addition, compounds from commercial sources can be tested, as well as commercially available analogs of identified inhibitors.

An agent may be a protein, a peptide, a DNA or RNA aptamer (e.g., a RNAi molecule), a lipid, or a small molecule (e.g., a lipid, carbohydrate, a bioinorganic molecule, or an organic molecule).

Agents that may be tested as a specific PARP activator include nucleic acids that contain a sequence encoding one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) domains of a PARP protein (e.g., a domain encoded by part of the nucleic acid sequence having at least 80% sequence identity to any one of SEQ ID NOS: 1-24).

Methods for Identification of an Agent that Specifically Binds One or More PARPs

The invention also provides methods for identifying an agent that specifically binds to one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 or more) PARP proteins and/or PARP fusion proteins. These methods require the contacting of one or more of the PARP proteins and/or PARP fusion proteins of the invention with a test agent and determining whether the test agent specifically binds to the one or more PARP proteins and/or PARP fusion proteins. An agent that specifically binds one or more of the described PARP proteins and/or PARP fusion proteins may act as an activator or inhibitor of the expression or activity of the one or more PARP proteins and/or PARP fusion proteins in a cell. For example, an agent that specifically binds to one or more PARP proteins and/or PARP fusion proteins may selectively increase the activity or expression of one or more PARP proteins and/or fusion proteins, while at the same time decreasing the activity or expression of one or more other PARP proteins and/or PARP fusion proteins in the same cell or sample.

The one or more PARP proteins and/or PARP fusion proteins used in this method may be attached to a solid surface or substrate (e.g., a bead) and/or may be present in purified form or present in a crude cell lysate, biological fluid, or extracellular medium. The methods may optionally include one or more (e.g., 1, 2, 3, 4, or 5) washing steps following contacting the one or more PARP proteins and/or PARP fusion proteins with the test agent. The test agent may be a small molecule, a lipid, an RNA molecule, a DNA molecule, a protein, or a peptide fragment. The test agent may be purified in form (e.g., at least 70%, 80%, 85%, 90%, 95%, or 99% pure by weight) or may be present in a crude cell lysate. The test agent may also, optionally be labeled (e.g., a colorimetric label, a radio-nuclide label, labeled with a biotin molecule, or labeled with a fluorophore).

The binding of the test agent to one or more PARP proteins and/or PARP fusion proteins may be detected by any known method including, BIAcore, competitive binding assays (e.g., a competitive binding assay using one or more of the antibodies provided by the invention), and detection of the agent following its release from the one or more PARP proteins and/or PARP fusion proteins (e.g., elution of the bound test agent following exposure to high salt or a high or low pH buffer). The one or more PARP proteins and/or PARP fusion proteins may be any of the example PARP proteins and PARP fusion proteins described herein.

In one example of this method, a bead attached to one or more PARP proteins and/or PARP fusion proteins of the invention (e.g., a ZZ-TEV-PARP fusion protein) may be incubated with a crude cell lysate, and the proteins or peptide fragments bound to the one or more PARP proteins and/or PARP fusion proteins may be eluted from the beads by expo-

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sure to a high salt buffer, a high detergent buffer, or a high or low pH buffer. The resulting eluted proteins may be electrophoresed onto an SDS-polyacrylamide gel and the specific protein bands cut out from the gel and analyzed using mass spectrometry to identify the specific agent that binds to the one or more PARP proteins and/or PARP fusion proteins.

In another example of the method, a bead attached to one or more PARP proteins and/or PARP fusion proteins of the invention is incubated with a purified protein or peptide fragment. In this instance, a protein or peptide fragment bound to the one or more PARP proteins and/or PARP fusion proteins may be eluted using a high salt buffer, a high detergent buffer, or a high or low pH buffer. The amount of protein in the eluate may be detected by any method known in the art including UV/vis spectroscopy, mass spectrometry, or any colorimetric protein dye (e.g., a Bradford assay).

In specific screening assays for agents that bind one or more PARP proteins and/or PARP fusion proteins, one or more PARP proteins and/or PARP fusion proteins may be placed in individual wells of a multi-well plate (e.g., one or more PARP proteins and/or PARP fusion proteins covalently linked to the plate surface) and incubated with the test agent. Following a washing step, the amount of test agent remaining in each well may be determined and the ability of the test agent to bind one or more PARP proteins and/or PARP fusion proteins determined.

The methods desirably identify a test agent that specifically binds one or more of a PARP1 protein and/or fusion protein, a PARP2 protein and/or fusion protein, a PARP5A protein and/or fusion protein, a PARP5B protein and/or fusion protein, a PARP7 protein and/or fusion protein, a PARP8 protein and/or fusion protein, a PARP14 protein and/or fusion protein, and a PARP16 protein and/or fusion protein of the invention. The methods also desirably identify a test agent that specifically binds to one or more of a PARP5A protein and/or fusion protein, a PARP12 protein and/or fusion protein, a PARP13.1 protein and/or fusion protein, a PARP13.2 protein and/or fusion protein, and a PARP15 protein and/or fusion protein of the invention. The methods also desirably identify a test agent that specifically binds to a PARP13.1 protein and/or fusion protein or a PARP11 protein and/or fusion protein of the invention.

Methods for Quantification of the Level of One or More PARPs in a Sample

The present invention further provides methods for quantitating the level of one or more PARP proteins or PARP fusion proteins present in a sample (e.g., a cell, a cell lysate, a biological fluid, or an extracellular medium). In these methods, a cell, cell lysate, biological fluid, or extracellular medium is contacted with one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) antibodies of the invention (e.g., antibodies that specifically bind to one or more PARP proteins or PARP fusion proteins described herein) and the level of one or more PARP proteins and/or PARP fusion proteins is determined by measuring the amount of the one or more PARP proteins and/or PARP fusion proteins bound to the one or more antibodies.

In these methods, the one or more antibodies may be polyclonal antibodies. The antibodies used in these methods may also be covalently bound to a bead (e.g., a magnetic bead or a bead in a column) or may be covalently bound to the surface of a multi-well plate (e.g., for use in an enzyme-linked immunosorbent assay (ELISA)). The quantitation of the binding of the one or more antibodies to the one or more PARP proteins and/or PARP fusion proteins may be determined by any method known in the art, including, but not limited to, BIA-core, immunofluorescence microscopy, immunofluores-

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cence-assisted cell sorting, ELISA, immunoblotting, and competitive binding assays (e.g., assays using purified labeled PARP proteins and/or PARP fusion proteins).

In these assays, the level of one or more PARP proteins and/or PARP fusion proteins may be compared to a standard curve control generated using one or more purified PARP proteins and/or PARP fusion proteins as described herein. The level of one or more PARP proteins present in a cell, cell lysate, or biological sample may be used as an indicator of the status or severity of one or more stress granule-related condition (e.g., a neurodegenerative disease, a cardiovascular disease, an inflammatory disease, and ischemia-reperfusion injury). For example, an increase in the level of one or more of PARP5A, PARP12, PARP13.1, PARP13.2, and PARP15 indicates an increased severity or an increase in the likelihood of developing a stress granule-related disorder.

Agent Screening Assays

The invention further provides methods for identifying a candidate agent for treating or decreasing a stress granule-related disorder requiring the steps of: providing one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARP, PARG, and/or ARH3 proteins, and/or PARP, PARG, and/or ARH3 fusion proteins encoded by a nucleic acid containing a nucleic acid sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to PARP5A (SEQ ID NO: 8 or 9), PARP12 (SEQ ID NO: 18), PARP13.1 (SEQ ID NO: 19), PARP13.2 (SEQ ID NO: 20), PARP15 (SEQ ID NO: 22 or 23), PARG (SEQ ID NO: 42), or ARH3 (SEQ ID NO: 41); contacting the one or more PARP, PARG, and/or ARH3 proteins, and/or PARP, PARG, and/or ARH3 fusion proteins with the agent and a labeled NAD⁺ substrate; and measuring the activity of the one or more PARP, PARG, and/or ARH3 proteins and/or PARP, PARG, and/or ARH3 fusion proteins or the specific binding of the agent to the one or more PARP fusion proteins; wherein an agent that decreases (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100%) the activity of the one or more PARP proteins and/or one or more PARP fusion proteins, and/or specifically binds to the one or more PARP proteins or fusion proteins, and/or increases the activity of the one or more PARG and/or ARH3 proteins or fusion proteins is identified as an agent for treating or decreasing the likelihood of developing a stress granule-related disorder.

The invention further provides methods for identifying a candidate agent for treating or decreasing the likelihood of developing cancer requiring the steps of: providing one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARP proteins and/or PARP fusion proteins encoded by a nucleic acid containing a nucleic acid sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or even 100%) to a PARP selected from PARP1 (SEQ ID NO: 1 or 2), PARP2 (SEQ ID NO: 3), PARP5A (SEQ ID NO: 8 or 9), PARP5B (SEQ ID NO: 10), PARP7 (SEQ ID NO: 12), PARP8 (SEQ ID NO: 13), PARP14 (SEQ ID NO: 21), or PARP16 (SEQ ID NO: 24); contacting the one or more PARP proteins and/or PARP fusion proteins with the agent and a labeled NAD⁺ substrate; and measuring the activity of one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) PARP proteins and/or PARP fusion proteins; wherein an agent that decreases (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100%) the activity and/or specifically binds to the one or more PARP proteins and/or PARP fusion proteins is identified as a candidate agent for treating or decreasing the likelihood of developing cancer.

In all the above screening methods, the one or more PARP, PARG, and/or ARH3 proteins, and/or PARP, PARG, and/or ARH3 fusion proteins may be generated using any methods

known in the art or the methods described herein. The PARP, PARG, and/or ARH3 proteins, and/or PARP, PARG, and/or ARH3 fusion proteins may be purified or present in a crude cell lysate. The PARP, PARG, and/or ARH3 proteins, and/or PARP, PARG, and/or ARH3 fusion proteins may be attached to a substrate (e.g., a magnetic bead) or a solid surface (multi-well plate).

The methods of the assay include the measurement of the activity of the one or more PARP, PARG, and/or ARH3 proteins, and/or PARP, PARG, and/or ARH3 fusion proteins using any assay known in the art or those assays described herein. The assays may include the incubation of the one or more PARP, PARG, and/or ARH3 proteins, and/or PARP, PARG, and/or ARH3 fusion proteins with a labeled NAD⁺ substrate and/or the incubation with other substrates that are not labeled (e.g., non-labeled NAD⁺). Each PARP, PARG, and/or ARH3 protein, and/or PARP, PARG, and ARH3 fusion protein may have one or more activities (described herein). Any known activity of a PARP, PARG, and/or ARH3 protein, and/or PARP, PARG, and/or ARH3 fusion protein (or activity described herein) may be measured in the above screening assays (e.g., catalytic addition of an ADP-ribose molecule or polymerization of a poly-ADP-ribose molecule, catalytic degradation of poly-ADP-ribose or removal of a ADP-ribose moiety from a substrate, localization to a particular cell structure (e.g., nucleus or stress granule), formation of a stress granule, disassembly of a stress granule, nucleation of a stress granule, promotion of cell proliferation or cell division, or required for cell cycle progression (e.g., progression through cytokinesis or mitosis)).

The ability of an agent to bind to one or more PARP or PARP fusion proteins may be measured using any method known in the art, as well as those methods described herein. For example, the ability of an agent to specifically bind to a PARP protein or PARP fusion protein may be measured by chromatography (e.g., using beads wherein a PARP protein, a PARP fusion protein, or a domain of a PARP protein or PARP fusion protein is covalently attached), BIAcore analysis, UV/vis spectroscopy, or calorimetry.

A variety of different agents may be tested in the above-described screening assays provided by the invention. For example, a candidate agent may be derived from or present in a crude lysate (e.g., a lysate from a mammalian cell or plant extract) or be derived from a commercially available chemical libraries. Large libraries of natural product or synthetic (or semi-synthetic) extracts or chemical libraries are commercially available and known in the art. The screening methods of the present invention are appropriate and useful for testing agents from a variety of sources for activity as a candidate agent. The initial screens may be performed using a diverse library of agents, but the method is suitable for a variety of other compounds and compound libraries. Such compound libraries can also be combinatorial libraries. In addition, compounds from commercial sources can be tested, as well as commercially available analogs of identified inhibitors.

An agent may be a protein, a peptide fragment, a DNA or RNA aptamer (e.g., a RNAi molecule), a lipid, or a small molecule (e.g., a lipid, carbohydrate, a bioinorganic molecule, or an organic molecule).

Candidate agents that may be tested include nucleic acids that contain a sequence encoding one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) domains of a PARP protein (e.g., a domain encoded by part of the nucleic acid sequence having at least 80% sequence identity to any one of SEQ ID NOS: 1-24), nucleic acids that contain a sequence encoding one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) domains of a PARG protein or ARH3 (e.g., a domain encoded by part of the nucleic acid

sequence having at least 80% sequence identity to SEQ ID NO: 41 and 42), or any polypeptide encoded by these nucleic acids.

In any of the above screening assays, the NAD⁺ may be labeled with a radioisotope (e.g., ³²P) or fluorophore (e.g., fluorescein), or be biotinylated.

Methods for Increasing Cell Proliferation in a Cell

The invention further provides methods of increasing the proliferation of a cell or population of cells requiring contacting the cell or population of cells with an effective amount of one or more PARP activators. In these methods, the one or more (e.g., 1, 2, 3, 4, or 5) PARP activators preferably increase the expression (e.g., mRNA and/or protein) and/or one or more activities of a PARP1, PARP2, PARP5A, PARP5B, PARP7, PARP8, PARP14, and PARP16. In these methods, the expression may be an increase (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%) in the level of one or more nucleic acid sequence(s) containing a sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or even 100%) to PARP1 (SEQ ID NOS: 1 or 2), PARP2 (SEQ ID NO: 3), PARP5A (SEQ ID NOS: 8 or 9), PARP5B (SEQ ID NO: 10), PARP14 (SEQ ID NO: 21), or PARP16 (SEQ ID NO: 24), or one or more polypeptides encoded by one of these nucleic acids. The activity of one or more PARPs may be any activity described herein, including, but not limited to, poly-ADP-ribosylation of a target protein (e.g., a protein localized to the nucleus or mitotic spindle during cytokinesis, or a PARP protein) or is required for progression through mitosis or cytokinesis.

A PARP activator encompassed by these methods may include one or more nucleic acid sequences containing a sequence having at least 80% sequence identity (e.g., 85%, 90%, 95%, 99%, or even 100%) to one of SEQ ID NOS: 1-3, 8-10, 21, or 24.

The rate of proliferation may be increased in a mammalian cell or a plant cell using these methods. For example, the rate of proliferation of a primary cell (e.g., a cell used for cell replacement therapies) may be increased using these methods. In another example, the rate of proliferation of a plant cell may be increased using these methods. These methods include the introduction of xenogenous nucleic acids encoding a PARP activator to create a transgenic mammalian or plant cell or transgenic mammal or plant.

Methods for Increasing Stress Granule Formation in a Cell

The invention further provides methods of increasing (e.g., by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%) the number of stress granules in a cell or cell population requiring contacting the cell or population of cells with one or more (e.g., 1, 2, 3, 4, or 5) PARP activators, one or more (e.g., 1, 2, 3, 4, or 5) PARG inhibitors, or one or more (e.g., 1, 2, 3, 4, or 5) PARP11 inhibitors. In these methods, the increased number of stress granules may be the result of an increase in the rate of stress granule nucleation or formation and/or a decrease in the rate of stress granule breakdown or turnover.

In these methods, the one or more PARP activators preferably increase (e.g., at least 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%) the expression (e.g., mRNA and/or protein) and/or activity of a protein encoded by a nucleic acid sequence containing a sequence that is at least 80% identical (e.g., at least 85%, 90%, 95%, 99%, or even 100% identical) to PARP5A (SEQ ID NO: 8 or 9), PARP12 (SEQ ID NO: 18), PARP13.1 (SEQ ID NO: 19), PARP13.2 (SEQ ID NO: 20), or PARP15 (SEQ ID NO: 22 or 23). The activity of the one or more PARPs may be an increase in the poly-ADP-ribosylation of one or more (e.g., 1, 2, 3, 4, or 5)

target protein(s) (e.g., a protein localized in a stress granule, a protein involved in the formation or disassembly of a stress granule, and/or a PARP protein) or the nucleation and/or formation of a stress granule. Additional activities of a PARP protein are described herein. Examples of the PARP activators that may be used with these methods include, nucleic acid sequence containing a sequence having at least 80% identity (e.g., at least 85%, 90%, 95%, 99%, or even 100% identical) to any one of SEQ ID NOS: 8, 9, 18-20, 22, or 23.

In these methods, the one or more PARG inhibitors may selectively decrease (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100% decrease) in the expression (e.g., mRNA and/or protein) or activity of PARG or glycohydrolase ARH3. For example, one or more PARG inhibitors may decrease the level of one or more nucleic acid sequence(s) containing a sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 99%, or even 100% identity) to PARG (SEQ ID NO: 42) or ARH3 (SEQ ID NO: 41), or a polypeptide encoded by these nucleic acids. The activities of PARG protein or ARH3 glycohydrolase are described herein and include, without limitation, the hydrolysis of poly-ADP-ribose (e.g., poly-ADP-ribose attached to a protein, e.g., a protein localized in a stress granule, a protein involved in the formation or disassembly of a stress granule, and/or a PARP), the prevention of the assembly of a stress granule, or disassembly of a stress granule. Examples of PARG inhibitors that may be used in these methods include an antibody that specifically binds to a polypeptide or peptide fragment encoded by a nucleic acid sequence containing a sequence having at least 80% sequence identity (e.g., 85%, 90%, 95%, 99%, or even 100%) to PARG (SEQ ID NO: 42) or ARH3 (SEQ ID NO: 41), or an RNA aptamer that binds to one or more of these nucleic acid sequences. Specific examples of RNAi molecules that may target PARG and ARH3 are SEQ ID NOS: 34 and 35, and SEQ ID NOS: 36 and 37, respectively.

In these methods, the one or more PARP11 inhibitors may selectively decrease (e.g., at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or even 100%) the expression (mRNA and/or protein) and/or activity of PARP11. In different examples of these methods, the PARP11 inhibitor(s) may decrease the expression of one or more nucleic acid(s) containing a sequence having at least 80% sequence identity (e.g., at least 85%, 90%, 95%, 99%, or even 100%) to PARP11 (SEQ ID NO: 17), or decrease the expression of one or more polypeptides encoded by these nucleic acids. The activity of PARP11 may include poly-ADP-ribosylation of a target protein (e.g., a protein localized in a stress granule, a polypeptide involved in the formation or disassembly of a stress granule, and/or a PARP), prevention of the assembly of a stress granule, or disassembly of a stress granule. Examples of PARP11 inhibitors that may be used in these methods include an antibody that specifically binds to a polypeptide or peptide fragment encoded by a nucleic acid sequence containing a sequence having at least 80% sequence identity (e.g., 85%, 90%, 95%, 99%, or even 100%) to PARP11 (SEQ ID NO: 17), or an RNA aptamer that binds to one or more of these nucleic acid sequences. Specific examples of RNAi molecules that may target PARP11 are SEQ ID NOS: 91-98.

These methods may allow for the specific up-regulation of stress granule formation in cells in which increased resistance to toxic stress is desired (e.g., a plant cell (e.g., a transgenic plant cell) or a cultured mammalian cell (e.g., for cell replacement therapies).

EXAMPLES

The features and other details of the invention will now be more particularly described and pointed out in the following

examples describing preferred techniques and experimental results. These examples are provided for the purpose of illustrating the invention and should not be construed as limiting.

Example 1

Generation of PARP-GFP Fusion Proteins and Assays

Fusion proteins containing the sequence of each PARP and green fluorescent protein (GFP) were generated using the pEGFP-C1 vector (Invitrogen) (FIG. 4). For these experiments, the DNA sequences encoding each of PARP1 (SEQ ID NOS: 1 and 2), PARP3 (SEQ ID NOS: 4, 5, and 6), PARP4 (SEQ ID NO: 7), PARP5A (SEQ ID NOS: 8 and 9), PARP5B (SEQ ID NO: 10), PARP6 (SEQ ID NO: 11), PARP7 (SEQ ID NO: 12), PARP8 (SEQ ID NO: 13), PARP9 (SEQ ID NO: 14), PARP10 (SEQ ID NOS: 15 and 16), PARP11 (SEQ ID NO: 17), PARP12 (SEQ ID NO: 18), PARP13.1 (SEQ ID NO: 19), PARP13.2 (SEQ ID NO: 20), PARP14 (SEQ ID NO: 21), PARP15 (SEQ ID NOS: 22 and 23), and PARP16 (SEQ ID NO: 24) were cloned into the pEGFP-C1 vector using the restriction sites indicated in Table 2. Each resulting plasmid contained a nucleic acid sequence encoding a PARP-GFP fusion protein, wherein the nucleic acid sequence encoding GFP was located 5' to the nucleic acid sequence encoding the PARP protein.

TABLE 2

Restriction Sites Used for Cloning PARP Sequences into pEGFP-C1	
PARP	Restriction Sites
1	BglII, SalI
3	BglII, SalI
4	KpnI, ApaI
5a	HinDIII, BglII
5b	SalI, BamHI
6	SalI, XmaI
7	BspEI, EcoRI
8	BspEI, SalI
9	BspEI, SalI
10	BamHI, BglII
11	SalI, BamHI
12	SalI, ApaI
13 isoform 1	BspEI, BamHI
13 isoform 2	BglII, BamHI
14	XhoI, SacII
15	SalI, BamHI
16	BglII, SalI

Each generated pEGFP-C1 vector was transfected into HeLa Kyoto cells using Lipofectamine 2000, according to the manufacturer's instructions. Cell lysate was prepared from the HeLa cells at 48 hours following transfection. Electrophoresis was performed on the cell lysate using 4-12% SDS-PAGE, and immunoblotting was performed using a rabbit anti-GFP polyclonal antibody (FIG. 5).

The localization of each PARP-GFP fusion protein in the transfected HeLa Kyoto cells was determined using immunofluorescence microscopy using rabbit anti-GFP polyclonal antibody and a fluorescently-labeled secondary antibody (FIG. 6). The data from this experiment indicate that several PARP-GFP proteins are primarily localized in the nucleus of asynchronous cells, including PARP1-GFP, PARP2-GFP, PARP7-GFP, and PARP8-GFP. The data further indicate that several PARP-GFP fusion proteins are localized in primarily in the cytoplasm of asynchronous cells, including PARP12-GFP, PARP13-GFP, PARP14-GFP, PARP15-GFP, PARP16-

GFP, PARP10-GFP, PARP11-GFP, PARP5A-GFP, and PARP5B-GFP. In addition, the data indicate that several PARP-GFP fusion proteins are localized in both the cytoplasm and the nucleus of asynchronous cells, including PARP3-GFP, PARP9-GFP, PARP6-GFP, and PARP4-GFP. The same pattern of cell localization for each PARP-GFP fusion protein was observed in the hTERT-RPE1 cell line (Clontech), a telomerase-immortalized human retinal pigment epithelial (RPE) normal cell line (FIG. 7).

Antibodies specific for each PARP were generated by immunizing rabbits with PARP-specific peptides conjugated to keyhole limpet hemocyanin (KLH) using known methods. The antibodies produced in the rabbit serum were later affinity purified using peptide columns (e.g., columns containing, as substrate, the specific peptide sequence used to inoculate the rabbit).

The antibodies for each PARP and a secondary-fluorescently labeled anti-rabbit polyclonal antibody were used to visualize the location of each PARP in asynchronous HeLa Kyoto cells transfected with a pEGFP-C1 plasmid encoding a PARP-GFP fusion protein (FIG. 8). The data from this experiment confirm that PARP1, PARP2, PARP7, PARP8, and PARP14 are primarily localized in the nucleus of asynchronous cells. The data from this experiment also confirm that PARP3, PARP4, PARP6, PARP9, and PARP15 are localized in the both the nucleus and the cytoplasm of asynchronous cells.

The localization of each PARP-GFP fusion protein (described above) was also determined in HeLa Kyoto cells transfected with a pEGFP-C1 plasmid encoding a PARP-GFP fusion protein following 12-hour treatment with 100 nM nocodazole or 5 µg/mL aphidicolin. Cells treated with nocodazole are arrested in S phase, while cells treated with aphidicolin are arrested in mitosis. FIG. 9 shows the cellular localization for each PARP-GFP fusion protein following cell arrest in S-phase or mitosis. The data show that the PARP1-GFP, PARP2-GFP, and PARP8-GFP fusion proteins localize to the nucleus during S-phase, and that PARP5A-GFP and PARP5B-GFP localize to the mitotic spindle during mitosis. The localization of these PARP-GFP fusion proteins (e.g., PARP1-GFP, PARP2-GFP, PARP5A-GFP, PARP5B-GFP, and PARP8-GFP) to the nucleus during S-phase and mitosis indicate a role for these PARP proteins in cell division and cell proliferation.

In order to study the role of PARP16, additional experiments were performed using RNAi knockdown of endogenous PARP16 or overexpression of PAPR16-GFP fusion proteins to study the effect of PARP16 knockdown and overexpression, respectively on cell morphology. Asynchronous HeLa Kyoto cells overexpressing PARP16-GFP protein had normal cell morphology (FIG. 10; middle panel). In these cells, the PARP16-GFP protein was primarily localized in the endoplasmic reticulum, as demonstrated by its co-localization with calnexin (FIG. 10; middle panel). HeLa Kyoto cells transfected with an RNAi molecule specific for PARP16 demonstrated significant morphological changes, including cell shrinkage and dramatic membrane defects (FIG. 10; right panel).

The specific cellular localization of each PARP-GFP fusion protein may be further analyzed by immunofluorescence microscopy using a combination of labeled antibodies specific for the GFP-tag of each PARP-GFP fusion protein and one or more markers of cellular structures or organelles. For example, immunofluorescence staining of asynchronous HeLa Kyoto cells transfected with a pEGFP-C1 vector expressing the PARP7-GFP fusion protein shows co-localization of an anti-GFP antibody and an anti-coilin antibody (a

marker of Cajal bodies in the nucleus) (FIG. 11). In another example, asynchronous HeLa Kyoto cells transfected with a pEGFP-C1 vector expressing the PARP16-GFP fusion protein shows co-localization of an anti-GFP antibody and an anti-calnexin antibody (a marker of the endoplasmic reticulum) (FIG. 10). A non-limiting list of marker proteins that may be used to determine the cellular localization of a PARP-GFP fusion protein is also provided in FIG. 11.

Experimental Methods

Kyoto HeLa cells were grown in DMEM supplemented with 10% FCS and penicillin/streptomycin at 37° C. in 5% CO₂. Lipofectamine 2000 (Invitrogen) was used to transfect the cells with each pEGFP-C1 vector according to the manufacturer's protocol. Cells were arrested in mitosis and S-phase by treatment with 100 nM nocodazole or 5 µg/mL aphidicolin for 12 hours, respectively. For immunofluorescence imaging, cells on coverslips were fixed in ice-cold methanol for five minutes and rehydrated in phosphate buffered saline (PBS). The cells were blocked in PBS containing 4% bovine serum albumin (BSA) and 0.1% Triton-X 100. All antibodies used for imaging were diluted in blocking buffer. The coverslips were incubated with primary antibodies for 45 minutes and with secondary antibodies for 30 minutes. Images were collected on a Nikon TE2000 confocal microscope equipped with a Yokogawa CSU-X1 spinning disk head, Hamamatsu ORCA ER digital camera, and NIS-Elements imaging software.

Example 2

Generation of ZZ-TEV-PARP Fusion Proteins

Fusion proteins containing the sequence of each PARP, a ZZ-domain of SEQ ID NO: 27, and four TEV protease recognition sequences (SEQ ID NO: 26) were cloned using the pcDNA3.1 vector (SEQ ID NO: 33) (Invitrogen) (FIG. 12) to yield a ZZ-4x-TEV-PARP fusion protein for each PARP. For these experiments, the DNA sequences encoding PARP1 (SEQ ID NOS: 1 and 2), PARP2 (SEQ ID NO: 3), PARP3 (SEQ ID NOS: 4, 5, and 6), PARP4 (SEQ ID NO: 7), PARP5A (SEQ ID NOS: 8 and 9), PARP6 (SEQ ID NO: 11), PARP7 (SEQ ID NO: 12), PARP9 (SEQ ID NO: 14), PARP10 (SEQ ID NOS: 15 and 16), PARP11 (SEQ ID NO: 17), PARP13.1 (SEQ ID NO: 19), PARP13.2 (SEQ ID NO: 20), PARP14 (SEQ ID NO: 21), PARP15 (SEQ ID NOS: 22 and 23), and PARP16 (SEQ ID NO: 24) were cloned into the pcDNA3.1 vector using the restriction sites indicated in Table 3. The sequence encoding the ZZ-domain and the sequence encoding the four TEV protease recognition sequences were cloned into the NheI and HindIII restriction sites in pcDNA3.1.

TABLE 3

Restriction Sites Used for Cloning PARP Sequences into pcDNA3.1	
PARP	Restriction Sites
1	XhoI, PmeI
2	BamHI, NotI
3	EcoRV, NotI
4	KpnI, ApaI
5a	HindIII, XhoI
6	EcoRV, NotI
7	BamHI, NotI
9	EcoRV, NotI
10	HindIII, XbaI
11	BamHI, XbaI
13 isoform 1	KpnI, BamHI

TABLE 3-continued

Restriction Sites Used for Cloning PARP Sequences into pcDNA3.1	
PARP	Restriction Sites
13 isoform 2	BamHI, EcoRV
14	KpnI, XhoI
15	KpnI, XhoI
16	KpnI, XbaI

Each resulting plasmid contained a nucleic acid sequence encoding a ZZ-TEV-PARP fusion protein, wherein the nucleic acid sequence encoding ZZ domain was located 5' to the nucleic acid sequence encoding the four TEV protease recognition sequences, which in turn, was located 5' to the nucleic acid sequence encoding each PARP.

Nucleic acids encoding each ZZ-TEV-PARP fusion protein may be transfected into target cells (e.g., HeLa Kyoto or HeLa S3 cells) and the resulting ZZ-TEV-PARP fusion proteins purified by binding to magnetic beads coated with a protein containing an Fc domain (e.g., IgG). The resulting ZZ-TEV-PARP fusion proteins may be used in the assays described below for the PARP-GFP fusion proteins and the other assays described herein. Assays utilizing the ZZ-TEV-PARP fusion proteins have the additional advantage of containing an engineered TEV protease recognition sequence, whereby the polypeptide tag on each PARP fusion protein (e.g., the ZZ-domain and the four TEV protease recognition sequences) may optionally be removed from the ZZ-TEV-PARP fusion proteins by treatment with TEV protease. In one example, one or more ZZ-TEV-PARP fusion proteins may be removed from a magnetic bead, resin, or solid surface by treatment with a TEV protease.

Example 3

PARP Activity Assays and Screening Methods

The above-described PARP fusion proteins may be used in PARP activity assays and in assays to identify an activator or inhibitor for a specific PARP or a specific subset of PARPs. An example of such an activity assay is shown in FIG. 13. In this example, cell lysate is first prepared from a HeLa S3 cell culture expressing one or more PARP-GFP fusion proteins. The cell lysate is then incubated with an anti-GFP polyclonal antibody bound to Dynabead® Protein A beads, and the beads magnetically removed from the cell lysate. The isolated beads bound to one or more PARP-GFP fusion proteins are placed into a multi-well plate and incubated with a labeled NAD⁺ substrate (e.g., ³²P-NAD⁺). Following incubation with the labeled NAD⁺ substrate, the magnetic beads bound with the one or more PARP-GFP proteins are magnetically isolated or washed, and the level of the label (i.e., the label present in the labeled NAD⁺ substrate) that is covalently attached to the one or more PARP-GFP fusion proteins bound to the magnetic beads is determined (e.g., the amount of ³²P covalently bound to the one or more PARP-GFP proteins attached to the beads). This assay provides a means of measuring the auto-modulation activity of one or more PARP-GFP fusion proteins (e.g., the ability of a PARP to modify its own structure by catalyzing the covalent attachment of one or more ADP-ribose molecules). The assay may also be designed such that lysate or PARP-GFP fusion proteins isolated from several different transfected HeLa S3 cells, each expressing a different PARP-GFP fusion proteins or subset of PARP-GFP fusion proteins, may be placed in different wells of the multi-well plate. The

assay may also be designed such that the lysate from several different transfected HeLa S3 cells is combined, wherein the lysate from each transfected HeLa S3 cell culture contains one or more PARP-GFP fusion proteins. In a different version of the assay, the PARP-GFP fusion proteins may contain a protease recognition site. In this version of the assay, the one or more PARP-GFP fusion proteins bound to the magnetic beads may be treated with a specific protease (i.e., a protease that recognizes a protease recognition sequence in the PARP-GFP fusion protein) to mediate release of the PARP-GFP fusion protein from the magnetic bead.

FIG. 14 provides an example of the use of the PARP-GFP fusion proteins of the invention for the identification of an agent that specifically inhibits the activity of one or more PARPs. This assay is similar to the assay described above, except that the one or more PARP-GFP fusion proteins is incubated with both a test agent and a labeled NAD⁺ substrate. A specific PARP inhibitor will decrease the amount of the label (i.e., the label present in the labeled NAD⁺ substrate) covalently attached to the one or more PARP-GFP fusion proteins bound to the magnetic beads relative to the amount of the label attached to the one or more PARP-GFP fusion proteins in the absence of the test agent. In different examples of this assay, lysate or PARP-GFP fusion proteins isolated from two or more different transfected HeLa S3 cells, each expressing a different PARP-GFP fusion protein or subset of PARP-GFP fusion proteins, may be placed in different wells of the multi-well plate. The assay may also be designed such that the lysate from several different transfected HeLa S3 cells is combined, wherein the lysate from each transfected HeLa S3 cells contains one or more PARP-GFP fusion proteins. The assay may also be specifically designed to identify inhibitors of a specific PARP-GFP protein or subset of PARP-GFP proteins including the subsets of: one or more of PARP1-GFP, PARP2-GFP, PARP5A-GFP, PARP5B-GFP, PARP7-GFP, PARP8-GFP, PARP14-GFP, and PARP16-GFP; one or more of PARP5A-GFP, PARP12-GFP, PARP13.1-GFP, PARP13.2-GFP, PARP15-GFP; PARP11-GFP; or PARP13.1-GFP.

Similar to the examples, described above, the PARP-GFP fusion proteins of the invention may be used to identify activators of one or more specific PARPs. In this instance, the assay may be used to identify agents that increase the amount of the label (i.e., the label present in the labeled NAD⁺ substrate) covalently attached to the one or more PARP-GFP fusion proteins bound to the magnetic beads relative to the amount of the label covalently attached to the one or more PARP-GFP fusion proteins in the absence of the test agent. Preferably, this assay may be designed to identify activators of a specific PARP-GFP fusion protein or subset of PARP-GFP fusion proteins including the subsets of: one or more of PARP1-GFP, PARP2-GFP, PARP5A-GFP, PARP5B-GFP, PARP7-GFP, PARP8-GFP, PARP14-GFP, and PARP16-GFP; one or more of PARP5A-GFP, PARP12-GFP, PARP13.1-GFP, PARP13.2-GFP, and PARP15-GFP; PARP11-GFP; or PARP13.1-GFP.

Example 4

Involvement of PARPs in Stress Granule Formation and Disassembly

We have discovered through a PARP family-wide RNAi screen that several PARP proteins are involved in the cell cycle and are required for progression through mitosis (e.g., PARP16). The identity of the various substrate proteins of the different PARP proteins remains largely unknown. To further

identify PARP substrate proteins and/or proteins that bind to poly-ADP-ribose polymers, the Bio-Gel P-6 resin shown in FIG. 15 was used to purify proteins that bind poly-ADP-ribose polymer and/or act as an acceptor of a ADP-ribose molecule or a poly-ADP-ribose polymer. FIG. 15 also shows a Coomassie Blue-stained SDS-PAGE gel showing the proteins present in the HeLa Kyoto cell extract (Extract), in cell extract following lectin clarification (Lectin Clarification), in the lysate prior to passing over the Bio-Gel P-6 resin (Input), in the pellet following centrifugation of the resin (Pellet), and in the eluate following treatment with poly-ADP-ribose glycohydrolase ARH3 (ARH3 Release). The data in FIG. 15 demonstrates the selective purification of proteins bound to the Bio-Gel P-6 resin.

We have discovered that poly-ADP-ribose polymers are associated with stress granules in cells during exposure to stress conditions. FIG. 16 shows the co-localization of poly-ADP-ribose polymers and eIF3, a marker of stress granules, in HeLa Kyoto cells following treatment with 0 or 250 μ M sodium arsenite for 30 minutes and immunostaining with fluorescently-labeled antibodies specific for poly-ADP-ribose polymers and eIF3. The data indicate that stress granules contain proteins modified with poly-ADP-ribose polymers.

In order to identify the specific PARP proteins that mediate the formation of the poly-ADP-ribose polymers present in stress granules, experiments were performed to determine whether the different PARP-GFP fusion proteins localize to stress granules. In these experiments, HeLa Kyoto cells transfected with a pEGFP-C1 plasmid expressing a PARP-GFP fusion protein were visualized using fluorescently-labeled anti-GFP and anti-eIF3 antibodies following treatment with 250 μ M sodium arsenite for 30 minutes (FIG. 17). The data indicate that the PARP5A-GFP, PARP12-GFP, PARP13.1-GFP, PARP13.2-GFP, and PARP15-GFP fusion proteins localize to stress granules under stress conditions.

Endogenous PARP5A, PARP12, PARP13/13.1, PARP15, and poly-ADP-ribose glycohydrolase (PARG) also localize to stress granules in HeLa Kyoto cells following treatment with 250 μ M sodium arsenite for 30 minutes (FIG. 18). In these experiments, the fixed cells were visualized using antibodies specific for one of PAR5A, PARP12, PARP13/13.1, PARP15, or PARG, and an anti-eIF3 antibody, and secondary fluorescently-labeled antibodies. The data indicate that PARG, as well as the endogenous- and fusion protein-forms of PARP5A, PARP12, PARP13/13.1, and PARP15, localize to stress granules under stress conditions. In a similar set of experiments using hTERT RPE cells, endogenous PARP5A, PARP12, PARP13, and PARP15 showed a similar cellular localization following exposure to 250 μ M sodium arsenite for 30 minutes, as was observed in HeLa Kyoto cells (FIG. 19).

Experiments using time-lapse immunofluorescence microscopy in live HeLa Kyoto cells further indicate that endogenous PARP12, PARP12-GFP, endogenous PARP13, and PARP13-GFP localize to stress granules at an early point in stress granule assembly and therefore, may play a regulatory role in the formation of stress granules (data not shown).

In an additional set of experiments, the effect of PARP13.1, PARP13.2, and PARP15 on stress granule formation was further studied by measuring the effect of overexpression of PARP13.1-GFP, PARP13.2-GFP, and PARP15-GFP on stress granule formation. In these experiments, HeLa Kyoto cells were transfected with a pEGFP-C1 plasmid encoding PARP13.1-GFP, PARP13.2-GFP, or PARP15-GFP. The transfected cells were stained with anti-GFP antibodies, anti-eIF3 antibodies, and fluorescently-labeled secondary antibodies. These data indicate that overexpression of PARP13.1-

GFP, PARP13.2-GFP, or PARP15-GFP fusion protein nucleates stress granule formation (FIG. 20).

In contrast to the effect mediated by overexpression of PARP13.1-GFP, PARP13.2-GFP, or PARP15-GFP fusion protein, overexpression of PARP11-GFP in HeLa Kyoto cells mediates a decrease in stress granule formation following treatment with 250 μ M sodium arsenite for 30 minutes (FIG. 21). In this experiment, HeLa Kyoto cells transfected with a pEGFP-C1 vector expressing a PARP11-GFP fusion protein were treated with sodium arsenite, and stained with anti-GFP antibodies, anti-eIF3 antibodies, and fluorescently-labeled secondary antibodies. These data indicate that overexpression of PARP11-GFP suppresses the formation of stress granules in cells exposed to stress conditions.

Experimental Methods

HeLa Kyoto cells were cultured as described above. Lipofectamine 2000 (Invitrogen) was used to transfect the HeLa Kyoto cells with a pEGFP-C1 plasmid encoding a PARP-GFP fusion protein (described above) according to the manufacturer's instructions. For stress granule induction, cells were treated with 250 μ M sodium arsenite for 30 minutes. For long-term, real-time imaging of PARP-GFP transfected HeLa cells, the cells were split into 24-well glass bottom plates and imaged every 20 minutes for 48 hours. Images were collected on a Nikon TE2000 confocal microscope equipped with a Yokogawa CSU-X1 spinning disc head, Hamamatsu ORCA ER digital camera, and NIS-Elements imaging software.

Example 5

Involvement of PARG and ARH3 in Stress Granule Disassembly

In order to determine the importance of poly-ADP-ribose polymers on stress granule formation and disassembly, an additional set of experiments were performed to test the effect of PARG and ARH3 activity on stress granule dynamics. In a first set of experiments, HeLa Kyoto cells were transfected with a pEGFP-C1 plasmid encoding PARG99-GFP, PARG102-GFP, or a PARG110-GFP fusion protein. Overexpression of PARG99-GFP, PARG102-GFP, or PARG110-GFP reduces the formation of stress granules in HeLa Kyoto cells following exposure to 100 μ M sodium arsenite for 30 minutes (FIG. 22). In these experiments, formation of stress granules was determined by staining the fixed cells with anti-eIF3 antibodies and secondary fluorescently-labeled antibodies. These data indicate that PARG activity (hydrolysis of poly-ADP-ribose) inhibits the formation of stress granules in cells under stress conditions.

Another set of experiments was performed to determine the effect of knockdown of PARG (SEQ ID NO: 42) or ARH3 (SEQ ID NO: 41) on stress granule formation in cells under stress conditions. In these experiments, HeLa Kyoto cells were treated with 30 nM siRNA specific for PARG (SEQ ID NOS: 34 and 35) or ARH3 (SEQ ID NOS: 36 and 37), or a control siRNA (All Stars Negative Control siRNA; Qiagen Catalog No. 1027280), and treated with 100 μ M sodium arsenite for 30 minutes, or 30 minutes or 1 hour following sodium arsenite washout (FIG. 23). Cells treated with a PARG siRNA or ARH3 siRNA show a sustained presence of stress granules following removal of sodium arsenite from the culture medium (via imaging using anti-eIF3 antibodies and fluorescently-labeled secondary antibodies). These data indicate that PARG and ARH3 activity (hydrolysis of poly-ADP-ribose) has a positive effect on stress granule disassembly, and that poly-ADP-ribose turnover kinetics regulate the formation/disassembly of stress granules. The percentage of

cells with stress granules following 30-minute washout and 1-hour washout after arsenite treatment was quantitated for cells treated with control siRNA, PARG siRNA, and ARH3 siRNA (FIG. 24). These data indicate that knockdown of PARG and ARH3 reduces the rate of stress granule disassembly following removal of the stress condition (sodium arsenite).

Experimental Methods

HeLa Kyoto cells were cultured in medium as described above. In PARG overexpression experiments, Lipofectamine 2000 (Invitrogen) was used to transfect HeLa Kyoto cells with pEGFP-C1 plasmids containing the nucleic acid sequences for each PARG isoform, i.e., PARG99, PARG102, and PARG110 (sequences described in Meyer-Ficca et al., *Exp. Cell. Res.* 297(2):521-532, 2004) according to the manufacturer's instructions. In PARG knockdown experiments, cells were treated with 30 nM of a siRNA targeting PARG (SEQ ID NOS: 34 and 35), a siRNA targeting ARH3 (SEQ ID NO: 36 and 37), or a control siRNA (AllStars Negative Control siRNA; Qiagen Catalog No. 1027280) using Lipofectamine 2000 according to the manufacturer's instructions. In these experiments, stress granule formation was induced by treatment with 100 μ M sodium arsenite for 30 minutes. For stress granule disassembly experiments, the media was replaced after sodium arsenite treatment, and cells were incubated for 30 minutes and 1 hour prior to fixation and immunostaining. At least 200 cells were counted for each condition (in triplicate) to determine the percentage of cells containing stress granules.

Example 6

Stress Granule Proteins Bind to GFP-PARPs

Experiments were performed to further identify stress granule-related proteins that may bind and be the substrates of one or more of the PARPs localized in stress granules (e.g., PARP5A, PARP12, PARP13, PARP13.1, and PARP15). In these experiments, HeLa S3 cells were transfected with pEGFP-C1 plasmids containing a nucleic acid sequence encoding PARP5A-GFP, PARP12-GFP, PARP13-GFP, PARP13.1-GFP, or PARP15-GFP fusion protein and treated with 0 or 250 μ M sodium arsenite for 30 minutes. The resulting cell lysate was immunoprecipitated using anti-GFP antibodies and the resulting immunoprecipitated proteins were electrophoresed using SDS-PAGE. The resulting gel indicates that each PARP-GFP fusion protein binds to several proteins and that treatment with sodium arsenite results in an alteration in the amount and identity of the proteins binding to each PARP-GFP fusion protein (FIG. 25A). In a similar experiment, the immunoprecipitated proteins are transferred to a membrane and immunostained with an anti-poly-ADP-ribose antibody. The data in this experiment show that PARP5A-GFP, PARP12-GFP, PARP13-GFP, and PARP13.1-GFP fusion proteins bind to poly-ADP-ribosylated proteins (FIG. 25B).

Data from a separate set of experiments indicate that several stress granule-associated proteins bind to the PARP13-GFP, PARP12-GFP, and PARP5A-GFP fusion proteins. In these experiments, HeLa S3 cells were transfected with a pEGFP-C1 plasmid encoding a PARP13-GFP, PARP12-GFP, or PARP5A-GFP fusion protein and treated with 0 or 20 nM pateamine A for 30 minutes. Cell lysates from the cells were immunoprecipitated using an anti-GFP antibody and the immunoprecipitated proteins were electrophoresed using 4-12% SDS-PAGE. The resulting proteins were transferred to a membrane and immunoblotted using commercially-available

antibodies specific for different stress granule-associated proteins: Ago2, DDX6, LSM1, PABP, FMRP, eIF1A, eIF2 α , eIF3 η , eIF4A1, and eIF4E. The data indicate that the PARP13-GFP, PARP12-GFP, and PARP5A-GFP fusion proteins have the ability to interact with one or more of these stress granule-associated proteins under both normal (0 nM pateamine A) and stress conditions (30 nM pateamine A) (FIG. 25C).

An additional set of experiments was performed to determine whether one or more stress granule-associated proteins are poly-ADP-ribosylated. In these experiments, HeLa S3 cells were transfected with a pEGFP-C1 plasmid encoding a GFP fusion protein of TIA1, PABP, G3BP, or Ago2, and treated with 0 or 20 nM pateamine A for 30 minutes. Lysates from these cells were immunoprecipitated using anti-GFP antibodies and immunoblotted using an anti-poly-ADP-ribose antibody. The data show that several proteins bind the TIA1-GFP, PABP-GFP, G3BP-GFP, and Ago2-GFP fusion proteins in untreated (0 nM pateamine A) and treated (20 nM pateamine A) cells (FIG. 26A). In an additional experiment, the proteins that bind to endogenous G3BP and Ago2 proteins in 250 μ M sodium arsenite-treated HeLa S3 cells were also shown to be poly-ADP-ribosylated (FIG. 26B). In this experiment, cell lysates from untransfected HeLa S3 cells treated with 0 or 250 μ M sodium arsenite for 60 minutes were immunoprecipitated with anti-G3BP or anti-Ago2 antibodies and immunoblotted using an anti-poly-ADP-ribose antibody.

G3BP1, a stress granule-associated protein, was shown to be poly-ADP-ribosylated (FIG. 26C). In order to map the specific domain in G3BP1 that is modified by a poly-ADP-ribose polymer, GFP-fusion proteins of different truncation 250 μ M sodium arsenite for 60 minutes. The specific nucleic acid sequences encoding each G3BP1 truncation mutant are described in Tourriere et al. (*J. Cell Biol.* 160:823-831, 2003). The cell lysate from each cell sample was immunoprecipitated using anti-GFP antibodies and immunoblotted using an anti-poly-ADP-ribose antibody. The data demonstrate that poly-ADP-ribosylation of G3BP1 occurs within the RNA-recognition motif (RRM) domain ("D" in FIG. 26C). The RRM domain of G3BP1 is a domain that binds to RNA molecules. The poly-ADP-ribosylation of G3BP1 in the RRM domain is thought to regulate the RNA-binding activity of G3BP1.

TIA1, a stress granule-associated protein, was also shown to be poly-ADP-ribosylated (FIG. 26D). In order to determine whether TIA1 is poly-ADP-ribosylated in its RRM domain, GFP-fusion proteins of full-length TIA1 and a truncation mutant of TIA1 lacking its RRM domain (TIA1 Δ RRM) were expressed in HeLa S3 cells treated with 0 or 250 μ M sodium arsenite for 60 minutes. The specific nucleic acid sequences encoding the full-length TIA1 and the TIA1 Δ RRM truncation mutant are described in Kedersha et al. (*J. Cell Biol.* 151:1257-1268, 2000). The cell lysate from each cell sample was immunoprecipitated using anti-GFP antibodies and immunoblotting was performed using an anti-poly-ADP-ribose antibody. The data demonstrate that poly-ADP-ribosylation of TIA1 also occurs within its RNA-recognition motif (RRM) domain (FIG. 26D). The poly-ADP-ribosylation of TIA1 in its RRM domain is also thought to mediate an alteration in its RNA-binding activity.

Experimental Methods

Immunoprecipitation experiments to identify proteins binding to PARP5A-GFP, PARP12-GFP, PARP13-GFP, PARP13.1-GFP, and PARP15-GFP were performed using HeLa S3 cells transfected with a pEGFP-C1 plasmid containing a nucleic acid sequence encoding each respective PARP-GFP fusion protein following treatment with 0 or 20 nM

pateamine A for 30 minutes. In each experiment, the cell lysate is incubated with an anti-GFP antibody to immunoprecipitate proteins bound to each of the PARP-GFP fusion proteins using standard methods. The resulting immunoprecipitated proteins were electrophoresed on 4-12% SDS-PAGE gels, and either stained directly with Coomassie Blue or transferred onto a membrane and immunostained with one or more of the following antibodies: anti-poly-ADP-ribose, anti-Ago2, anti-DDX6, anti-LSM1, anti-PABP, anti-FMRP, anti-eIF1A, anti-eIF2 α , anti-eIF3 η , anti-eIF4A1, and anti-eIF4e antibodies.

Immunoprecipitation experiments using TIA1-GFP, PABP-GFP, G3BP-GFP, and Ago2-GFP fusion proteins were performed using HeLa S3 cells transfected with pEGFP-C1 plasmids containing a sequence encoding a nucleic acid sequence encoding TIA1 (Kedersha et al., *J. Cell Biol.* 151: 1257-1268, 2000), PABP (NCBI Accession No. NM_12154.2), G3BP (Tourriere et al., *J. Cell Biol.* 160:823-831, 2003), Ago2 (NCBI Accession No. U002568.3), a truncation mutant of G3BP (i.e., A, ABC, BC, BCD, and D truncation mutants described in Tourriere et al., supra), or the Δ RRM truncation mutation of TIA1 (described in Kedersha et al., supra) following treatment with 0 or 20 nM pateamine A for 30 minutes. In each experiment, the cell lysate is incubated with an anti-GFP antibody to immunoprecipitate proteins bound to each of the GFP fusion proteins using standard methods. The resulting immunoprecipitated proteins were electrophoresed on 4-12% SDS-PAGE gels, and either stained directly with Coomassie Blue or transferred onto a membrane and immunostained with anti-poly-ADP-ribose antibody.

Example 7

PARP13 and PARG Regulation of RNAi Activity

We have further discovered that PARP13 and PARG regulate the activity of RNAi and miRNA molecules in cells. Regulation of RNAi and miRNA activity in cells remains largely uncharacterized. One of the proteins implicated for a role in the regulation of RNAi and miRNA activity is Argonaut 2, a single-stranded RNase. Using immunofluorescence microscopy we have observed that Argonaut 2 localizes to stress granules in HeLa cells treated with 250 μ M sodium arsenite for 30 minutes (FIG. 27, left panel). In these experiments, cells were treated with sodium arsenite and stained using both antibodies against Argonaut 2 and eIF3 (a stress granule marker), and secondary fluorescently-labeled antibodies. The data show that Argonaut 2 is poly-ADP-ribosylated in HeLa cells following exposure to 250 μ M sodium arsenite for 30 minutes (FIG. 27, right panel). In these experiments, cell lysate from cells treated with 250 μ M sodium arsenite was immunoprecipitated with an anti-Argonaut 2 antibody, and the resulting immunoprecipitated proteins were immunoblotted using an anti-poly-ADP-ribose antibody. The results indicate that Argonaut 2 is localized to stress granules and poly-ADP-ribosylated under cellular stress conditions.

To determine whether one or more of the PARPs identified herein mediate the poly-ADP-ribosylation of Argonaut 2, immunoprecipitation experiments were performed on cell lysate from untransfected HeLa cells treated with 0 or 250 μ M sodium arsenite for 30 minutes using an anti-Argonaut 2 antibody. The resulting immunoprecipitated proteins were immunoblotted using an anti-PARP13/13.1 antibody. The data show that PARP13/13.1 binds to Argonaut 2 under both normal (0 μ M sodium arsenite) and stress conditions (250 μ M sodium arsenite) (FIG. 28).

To identify additional substrate proteins of PARP13, immunoprecipitation experiments were performed on lysate from HeLa cells transfected with a pEGFP-C1 plasmid containing a sequence encoding a PARP13-GFP fusion protein following treatment with either 0 or 250 μ M sodium arsenite for 30 minutes. The cell lysate was treated with an anti-GFP antibody and the resulting immunoprecipitated proteins were electrophoresed using SDS-PAGE. The data show that exposure to 250 μ M sodium arsenite increases the number and identity of proteins that bind to the PARP13-GFP fusion protein (FIG. 29). The identification of the specific proteins co-immunoprecipitated with the PARP13-GFP fusion protein will help to further elucidate the role of PARP13 in cellular mechanisms, including its regulation of Argonaut 2 and its role in the regulation of miRNA and RNAi activity. Additional experiments were performed to determine the effect of PARP13 knockdown on miRNA activity. For these experiments, the pGL4.72[hRlucCP]TM-vector assay (Promega) was used to measure RNAi activity in 293T cells. The pGL4.72[hRlucCP]TM vector contains a constitutively expressed firefly luciferase gene which is located upstream of several nucleic acid sequences targeted by an RNAi molecule. An increase in the activity of an RNAi molecule targeting the downstream 3' sequences of the vector results in a decrease in the amount of luciferase produced from the vector. In experiments to study the effect of PARP13 on miRNA activity, the pGL4.72[hRlucCP]TM vector was first engineered to contain 6 repeats of a sequence recognized by an RNAi molecule targeting the vector ("vector-target RNAi;" SEQ ID NOS: 38 and 39; GUUUUCACUCCAGCUAACACA and TTCAAAAGUGAGGUCGAUUGU, respectively). In a first experiment, cells were transfected with a modified pGL4.72[hRlucCP]TM vector and a pEGFP-C1 plasmid encoding EGFP (negative control), G3BP (negative control), PARP5a, PARP12, PARP13, PARP13.1, or PARP15; and 10 nM of the vector-target RNAi. In a positive control, the cells were transfected with the modified pGL4.72[hRlucCP]TM vector and vector-target RNAi alone (CXCR4 sponge). Cells overexpressing PARP13 or PARP13.1 showed a 3-fold decrease in the level of miRNA-mediated repression compared to control cells (e.g., EGFP- and G3BP-overexpressing cells) (FIG. 30). In a second set of experiments, the ability of the vector-target RNAi to reduce the expression of luciferase was measured in 293T cells transfected with pGL4.72[hRlucCP]TM vector, 20 nM vector-target RNAi, and 20 nM of negative RNAi control for PARP13 siRNA (siNeg; AllStars Negative Control siRNA; Qiagen Catalog No. 1027280) or PARP13 siRNA (siPARP13; SEQ ID NO: 40) following treatment with 0 or 30 nM pateamine A for 2 hours. The data in FIG. 31 show that knockdown of PARP13 expression by siPARP13 results in an increase in the activity of the vector-target RNAi under stress conditions (i.e., 30 nM pateamine A) (FIG. 31). These data indicate that PARP13 activity in the cell has a negative effect on RNAi activity in the cell. This effect on RNAi activity may occur through the poly-ADP-ribosylation of Argonaut 2 by PARP13 or by the ability of PARP13 to modify or bind other proteins located within stress granules or proteins required for the assembly or disassembly of stress granules.

Experimental Methods

Immunoprecipitation experiments were performed using non-transfected HeLa cells following treatment with 0 or 250 μ M sodium arsenite for 60 minutes using an anti-Argonaut 2 antibody. The resulting precipitated proteins were electrophoresed using 4-12% SDS-PAGE and immunoblotted using an anti-poly-ADP ribose antibody. Non-transfected HeLa cells treated with 250 μ M sodium arsenite for 30 minutes

were also stained for immunofluorescence microscopy using antibodies specific for Argonaut 2 and eIF3 (a marker of stress granules), and a secondary fluorescently-labeled antibody (Alexa Fluor 594 and 488 antibodies).

Additional co-immunoprecipitation experiments were performed using methods known in the art. In these experiments, HeLa cell lysate was prepared from cells treated with 0 or 250 μ M sodium arsenite for 30 minutes, and the lysate subsequently immunoprecipitated with an anti-Argonaut 2 antibody. The resulting precipitated proteins were immunoblotted using an anti-PARP13/13.1 antibody.

Experiments to identify additional proteins bound to a PARP13-GFP fusion protein were performed by transfecting HeLa S3 cells with a pEGFP-C1 plasmid encoding a PARP13-GFP fusion protein. The transfected cells were treated with 0 or 250 μ M sodium arsenite for 30 minutes before lysis. The resulting cell lysate was immunoprecipitated with an anti-GFP antibody and the resulting precipitated proteins were electrophoresed using 4-12% SDS-PAGE and the resulting gel stained with Coomassie Blue.

Experiments to determine the effect of knockdown of PARP13 on miRNA and RNAi activity were performed using a modified pGL4.72[hRlucCP]TM-vector assay (Promega). For these experiments, the pGL4.72[hRlucCP]TM-vector was modified by placing six copies of a target sequence at a position 3' to the luciferase gene. RNAi molecules targeting the vector were designed to bind the six copies of the target sequence (SEQ ID NOS: 34 and 35). The modified pGL4.72

[hRlucCP]TM-vector was introduced into 293T cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. In each experiment, the cells were further transfected (Lipofectamine 2000) with 10 or 20 nM of the vector-target RNAi alone or in combination with either 20 nM of a control RNAi molecule for the siRNA targeting PARP13 (siNeg) or an RNAi molecule targeting PARP13 (siPARG13), and the cells treated with 0 or 30 nM patemine A for 60 minutes. Following 48-hours incubation, the level of luciferase protein production was measured using a luciferase assay kit (Promega). The data are shown as the relative level of luciferase protein produced in cells transfected with the modified vector alone in the absence of any RNAi treatment.

Experiments were also performed to determine the effect of overexpression of a PARP-GFP protein on the activity of miRNA using the modified pGL4.72[hRlucCP]TM-vector assay described above. In these experiments, 293T cells were transfected with pEGFP-C1 expression vectors encoding EGFP, G3BP, PARP5A, PARP12, PARP13, PARP13.1, or PARP15; the modified pGL4.72[hRlucCP]TM vector, and 10 nM vector-targeting RNAi. As a positive control for RNAi activity, the cells were transfected with the modified pGL4.72 [hRlucCP]TM and the vector-target RNAi alone (CXCR4 sponge).

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention; can make various changes and modifications of the invention to adapt it to various usages and conditions.

SEQUENCE LISTING

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<211> LENGTH: 1089

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 2

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<211> LENGTH: 1716

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 3

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<211> LENGTH: 1605

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 4

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 <211> LENGTH: 1602
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens

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<210> SEQ ID NO 6
 <211> LENGTH: 1602
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 6

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<211> LENGTH: 5178

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 7

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<210> SEQ ID NO 8

<211> LENGTH: 3987

<212> TYPE: DNA

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<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

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<210> SEQ ID NO 9

<211> LENGTH: 729

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 9

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caaagtacta ttcagaaaca cagagatggg ggtaatgctg gcggcatctt caacagatac	180
aatgtcattc gaattcaaaa agttgtcaac aagaagttga gggagcgggt ctgccaccga	240
cagaaggaag tgtctgagga gaatcacaaac catcacaaat agcgcattgt gtttcatggg	300
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<211> LENGTH: 3504

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 10

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<210> SEQ ID NO 11

<211> LENGTH: 1563

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 11

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gaatatggaa ctatcgatga cgtggacatt gacctccaca tcaacatcag ctctctcgat 240
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<210> SEQ ID NO 12

<211> LENGTH: 1977

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 12

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agtcatggca tgagaaggcc cccgccagtc aatcctggca gtgtcaccag tgacctttat   1860
gactcttggt tggataattt ctttgagcct cagatttttg tcatttttaa tgatgaccag   1920
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<210> SEQ ID NO 13

<211> LENGTH: 2568

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 13

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acttttacct acgttggcgg ccccgagaag gtatccctact cagtacatgt atctgaagat   180
taccagata atacatatgt gtcaagttca gagaatgatg aagatgtgct agttactaca   240
gagccaatac cagtaatttt tcatagaata gcaacagaat taagaaaaac aaatgacatt   300
aactgttgct tatccataaa atccaaatta caaaaggaaa atggggagga atcaagacag   360
aatagtacag tggaggaaga ttctgaaggt gacaatgatt ccgaagaatt ttattacgga   420
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cacaaacttt tgaaccgtcc ttgccctgca gctgttaagt cagaggaatg cctaactcta 1140
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ttcgtctatg aagacggcca agtgggagat gcaaatatta atacacaaga aggaggcatt 2520
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<210> SEQ ID NO 14

<211> LENGTH: 2568

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 14

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gggaatacag aagaatgtct cccctacaag tgctcagaga ctggtgctct tggagaaaac 180
tatagttggc aaattcccat taaccacaat gacttcaaaa ttttaaaaaa taatgagcgt 240
cagctgtgtg aagtcctoca gaataagttt ggctgtatct ctaccctggt ctctccagtt 300
caggaaggca acagcaaatc tctgcaagtg ttcagaaaaa tgctgactcc taggatagag 360
ttatcagctt ggaaagatga cctcaccaca catgctgttg atgctgtggt gaatgcagcc 420
aatgaagatc ttctgcatgg gggaggcctg gccctggccc tggtaaaagc tgggtgattt 480
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attctgaatt atgtcatcta taaaaatact cacattaaga cagtagcaat tccagccttg	720
agctctggga tttttcagtt cctcttgaat ttgtgtacaa agactattgt agagactatc	780
cgggttagtt tgcaagggaa gccaatgatg agtaatttga aagaaattca cctggtgagc	840
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gagctgggac aagaaccac ccttctttc aatgcaatgg tegtgaacaa cctgaccctc	960
cagattgtcc agggccacat tgaatggcag acggcagatg taattgttaa ttctgtaaac	1020
ccacatgata ttacagtgg accgtggca aagtcaattc tacaacaagc aggagttgaa	1080
atgaaatcgg aatttcttgc cacaaggct aaacagtttc aacggcccc gttggctactg	1140
gtcacaaaag gatttaactt gttctgtaaa tatatatacc atgtactgtg gcattcagaa	1200
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caaaatataa cttccatttc ctttctgcc cttgggactg gaaacatgga aataaagaag	1320
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<210> SEQ ID NO 15

<211> LENGTH: 3081

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 15

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ggccgctccc cagacactta a	3081

<210> SEQ ID NO 16

<211> LENGTH: 3081

<212> TYPE: DNA

<213> ORGANISM: Homo Sapiens

<400> SEQUENCE: 16

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ggacctgtgt tgagctggca gagactgggc tgtggggcgg tcctcacctt cagagagcct	180
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<210> SEQ ID NO 17

<211> LENGTH: 999

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 17

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atgtttcagc cggataccaa cagtcagtgt tcagttagca gtgaagatat cgaaaaaagc	180
ttcaaaacaa acccttgtgg ctccatttct tttactactt ccaaattcag ctacaagata	240
gactttgcag aaatgaagca aatgaatctc accactggaa agcagcgctt aataaaaaga	300
gccccctttt ctatcagtgc ttccagttac atctgtgaaa acgaggccat cctatgcca	360
ccacactggg agaatgtgaa tactcaagta ccatatcagc ttattcctct gcacaatcaa	420
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aaaagaattc agagaattca aaacctagat ttgtgggagt tcttttgcag gaaaaaggct	540
cagctcaaga aaaaaagagg tgtgcctcag attaatgaac aaatgctgtt tcatggtacc	600
agcagtgaat ttgtggaagc aatctgcatt cataactttg attggagaat aaatggtata	660
catggtgctg tctttggaag aggaacctat ttgctagag atgctgctta ttccagtcgt	720
ttctgcaaag atgacataaa gcatgggaac acattccaaa ttcattggtgt cagcttgcaa	780
cagcggcatc tgtttagaac atataaatct atgtttcttg ctgcagtgc aattggagat	840
tacataaacg gagactccaa atacatgcga cctccttcca aagacgggag ctatgtgaat	900
ttatatgaca gctgtgtgga tgatacctgg aacccaaaga tctttgtggt tttgatgcc	960
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<210> SEQ ID NO 18

<211> LENGTH: 2109

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 18

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gagcggctgc tgccgcagcg tgggcgcttc gtggtggcgg tgccggcggg cgccgcagcc	180
gcggcccccg agcgcgtggt gctggccgcc tcgccgctgc gcctgtgtcg cgcgcaccag	240
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tacggcgctt gcaagttcct gagagccggg aagaactgta ggaatagtca cagcttgaca	360
accgaacaca acctgagtgt gctgagaact catggcgttg accacctgag ctataatgag	420
ctatgccaac tcttgtttca gaacgacccc tggcttttgc cagaaatttg ccaacattac	480
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gatttctcta attctgagaa tctggaaaaa ttggagaagt tgggtatgag ctacagacctg	660
gtgagcaggc tgccatccat ttatagaaat gcacatgaca tcaagaataa gagctctgcc	720
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cccaaatacg tgtctcccca gtagtgacg accatgcaaa cctgcaatac caagtttcca	1440
ggcccgaaga gcacccacga ctattgggac tctctgccc tgccagaccc aggttttcag	1500
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atctttgtga tctttgagaa acaccaggtc taccagagt atgtcatcca gtacaccacc	2040
tcctccaagc cctcggtcac accctccatc ctgctggcct tgggctccct gttcagcagc	2100
cgacagtga	2109

<210> SEQ ID NO 19

<211> LENGTH: 2712

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 19

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gtgctgcagg tggccggggc cgaccgcttt gtggtgtgg agaccggcgg cgaggccggg	180
atcacccgat cgggtggtgc caccactcga gcccgggtct gccgtcgcaa gtactgccag	240
agaccctgcg ataacctgca tctctgcaaa ctcaacttgc tgggccggtg caactattcg	300
cagtcagagc ggaatttatg caaatattct catgaggctc tctcagaaga gaacttcaaa	360
gtcctgaaaa atcacgaact ctctggactg aacaaagagg aattagcagt gctcctctc	420
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cagatttgta accagcagcc accgtgttca agactccaca tctgtgacca cttcaccgca	540
gggaactgtc gttttcccaa ctgcctccgg tcccataacc tgatggacag aaagggtctg	600
gccatcatga gggagcacgg gctgaacccc gacgtggtcc agaacatcca ggacatctgc	660
aacagcaagc acatgcagaa gaatcccca gggcccagag ctccctcttc acatcgtaga	720
aacatggcat atagggttag aagcaagagt agagatcggt tctttcaggg cagccaagaa	780
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ttgcatggaa atccaggcag cacttacctt gcttccaatt caacatcagc ccccaactgg	1080
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cctaggatcc aggatgctgg acctgcttcc cgagatgtcc aggccactgg cagaatcgca	1440
gatgatgctg acccaagagt agcacttgtt aacgattctt tatctgatgt cacaagtacc	1500
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gtgattagtt ag 2712

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<210> SEQ ID NO 20
<211> LENGTH: 2103
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 20

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gtgctgcagg tggccgggcc cgaccgcttt gtggtgttgg agaccggcgg cgaggccggg 180
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cagtccgagc ggaatttatg caaatattct catgagggtc tctcagaaga gaacttcaaa 360
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aacagcaagc acatgcagaa gaatccccca gggcccagag ctcttcttcc acatcgtaga 720
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ttgcatggaa	atccaggcag	cacttacctt	gcttccaatt	caacatcagc	ccccaactgg	1080
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gagctgagtt	tccaagggat	gattcagaca	aacatagctt	ccaaaactca	aaaggatgtc	2040
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<210> SEQ ID NO 21

<211> LENGTH: 4560

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 21

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gaagagtcac	tagatcttcc	cttatggaag	ttcttacaga	aaaagaatca	cctcattgag	180
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ttgattgagt	ttgatacact	taaggagatg	gtaatcttag	cagggaaatc	agaggatgtc	480
caaagcattg	aggtacaagt	caggagagta	atagaaagca	ctactcaaaa	aattaaaagg	540
gaagagcaaa	gtttgaagga	aaaaatgac	atttctccag	gcaggatatt	tcttttgtgt	600
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agagtcactc	aacacttggt	cttgaaagga	cctagtgcag	atgtgtataa	agcaaagtgt	720
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aagaacaaaa attcctcccc aaacactgta atcatcaatg agttaacttc agaaaccaca	1080
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<210> SEQ ID NO 22

<211> LENGTH: 2040

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 22

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cggagtatgt ccagagacaa caagttcagc aagaaagatt gtctttcaat caggaatgtt	240
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ccactatctc gggcattttt gcagaaagct ggtcccagc tccagaaaga gttagatgac	420
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cctttacaag aagtcactt tctggtatat acaaatgacg atgaaggctg tcaggcattt	780
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ggagataccc aaggtgtggt cgggactgtc tetaagcctt gtttcacagc atatgaaatg	900
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gttattgtaa actcaacagc aaggacattt aatcggaat caggtgtgtc aagagctatt	1020
ttagaaggtg ctggacaagc tgtggaagt gaatgtgtg tactagctgc acagcctcac	1080
agagatttta taattacacc aggtggatgc ttaaagtgc aaataataat tcatgttcct	1140
gggggaaaag atgtcaggaa aacggtcacc agtgttctag aagagtgtga acagaggaag	1200
tacacatcgg tttcccttcc agccattgga acaggaaatg ccggaaaaaa cctatcaca	1260
gttgctgata acataatcga tgctattgta gacttctcat cacaacattc caccocatca	1320
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tgtaatcttc ctgaacactg gactgacatg aatcatcagc tgttttgcac ggtccagcta	1500
gagccaggac aatcagaata taataccata aaggacaagt tcacccgaac ttgttcttcc	1560
tacgcaatag agaagattga gaggatacag aatgcatttc tctggcagag ctaccaggta	1620
aagaaaaggc aaatggatat caagaatgac cataagaata atgagagact cctcttccat	1680
gggacagatg cagactcagt gccatatgtc aatcagcagc gctttaatag aagttgtgct	1740
gggaaaaaatg ctgtatccta tggaaaagga acctattttg ctgtggatgc cagttattct	1800
gccaaaggaca cctactccaa gccagacagc aatgggagaa agcacatgta cgttgtgcga	1860
gtacttactg gagtcttcac aaagggacgt gcaggattag tcacccctcc acccaagaat	1920
cctcacatc ccacagatct ctttgactca gtgacaaaca atacacgac tcctaaagcta	1980
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<210> SEQ ID NO 23

<211> LENGTH: 1338

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 23

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ttctatttcc aggcattttt agatgaattc actaactggc caagaataaa tcccaacaag	120
gccaggattc ccattggcagg agatacccaa ggtgtggctg ggactgtctc taagccttgt	180
ttcacagcat atgaaatgaa aatcggtgca attacttttc aggttgctac tggagatata	240
gccactgaac aggtagatgt tattgtaaac tcaacagcaa ggacatttaa tcggaaatca	300
ggtgtgtcaa gagctatttt agaagggtgct ggacaagctg tggaaagtga atgtgtgtga	360
ctagctgcac agcctcacag agattttata attacaccag gtggatgctt aaagtgcaaa	420
ataataattc atgttcctgg gggaaaagat gtcaggaaaa cggtcaccag tgttctagaa	480
gagtgatgaac agaggaagta cacatcggtt tcccttccag ccattggaac aggaaatgcc	540
ggaaaaaacc ctatcacagt tgctgataac ataatcgatg ctattgtaga cttctcatca	600
caacattcca ccccatcatt aaaaacagtt aaagttgtca tttttcaacc tgagctgcta	660
aatatattct acgacagcat gaaaaaaga gacctctctg catcactgaa ctttcagtc	720
acattctcca tgactacatg taatcttcct gaacactgga ctgacatgaa tcatcagctg	780
ttttgcatgg tccagctaga gccaggacaa tcagaatata ataccataaa ggacaagtcc	840
acccgaactt gttcttccta cgcaatagag aagattgaga ggatacagaa tgcatctctc	900

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65	70	75	80
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Ile Gln Glu Arg			
	85	90	95
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Ser Arg Ala Glu Val			
	100	105	110
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Thr Gly Thr			
	115	120	125
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly Asn Lys Met Glu Tyr Asn			
	130	135	140
Tyr Asn Ala His Asn Val Tyr Ile Met Thr Asp Lys Ala Lys Asn Gly			
	145	150	155
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val			
	165	170	175
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro			
	180	185	190
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Thr Leu Ser			
	195	200	205
Lys Asp Pro Asn Glu Lys Arg Asp His Met Ile Tyr Phe Glu Phe Val			
	210	215	220
Thr Ala Ala Ala Ile Thr His Gly Met Asp Glu Leu Tyr Lys			
	225	230	235

<210> SEQ ID NO 26
 <211> LENGTH: 7
 <212> TYPE: PRT
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic Construct
 <220> FEATURE:
 <221> NAME/KEY: MISC_FEATURE
 <222> LOCATION: (2)..(2)
 <223> OTHER INFORMATION: Xaa is any amino acid
 <220> FEATURE:
 <221> NAME/KEY: MISC_FEATURE
 <222> LOCATION: (3)..(3)
 <223> OTHER INFORMATION: Xaa is any amino acid
 <220> FEATURE:
 <221> NAME/KEY: MISC_FEATURE
 <222> LOCATION: (5)..(5)
 <223> OTHER INFORMATION: Xaa is any amino acid

<400> SEQUENCE: 26

Glu Xaa Xaa Tyr Xaa Gln Ser
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<210> SEQ ID NO 27
 <211> LENGTH: 126
 <212> TYPE: PRT
 <213> ORGANISM: Staphylococcus aureus

<400> SEQUENCE: 27

Gly Pro Ser Ala Val Asp Asn Lys Phe Asn Lys Glu Gln Gln Asn Ala			
1	5	10	15
Phe Tyr Glu Ile Leu His Leu Pro Asn Leu Asn Glu Glu Gln Arg Asn			
	20	25	30
Ala Phe Ile Gln Ser Leu Lys Asp Asp Pro Ser Gln Ser Ala Asn Leu			
	35	40	45
Leu Ala Glu Ala Lys Lys Leu Asn Asp Ala Gln Ala Pro Lys Val Asp			
	50	55	60
Asn Lys Phe Asn Lys Glu Gln Gln Asn Ala Phe Tyr Glu Ile Leu His			
	65	70	75
			80

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Leu Pro Asn Leu Asn Glu Glu Gln Arg Asn Ala Phe Ile Gln Ser Leu
 85 90 95

Lys Asp Asp Pro Ser Gln Ser Ala Asn Leu Leu Ala Glu Ala Lys Lys
 100 105 110

Leu Asn Asp Ala Gln Ala Pro Lys Val Asp Ala Asn His Gln
 115 120 125

<210> SEQ ID NO 28
 <211> LENGTH: 4731
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 28

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gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc attgacgtca	180
atgggtggag tatttacggt aaactgcca cttggcagta catcaagtgt atcatatgcc	240
aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt atgccagta	300
catgacctta tgggactttc ctacttgga gtacatctac gtattagtca tcgctattac	360
catggtgatg cggttttggc agtacatcaa tgggcgtgga tagcggtttg actcacgggg	420
atttccaagt ctccacccca ttgacgtcaa tgggagtttg ttttggcacc aaaatcaacg	480
ggactttcca aaatgtcgta acaactccgc ccattgacg caaatgggcg gtaggcgtgt	540
acggtgggag gtctatataa gcagagctgg tttagtgaac cgtcagatcc gctagcgcta	600
ccggtcgcca ccatggtgag caagggcgag gagctgttca ccggggtggt gcccatcctg	660
gtcgagctgg acggcgacgt aaacggccac aagttcagcg tgtccggcga gggcgagggc	720
gatgccacct acggcaagct gacctgaag ttcctctgca ccaccggcaa gctgcccgtg	780
ccctggccca cctcgtgac cacctgacc tacggcgtgc agtgcttcag ccgctacccc	840
gaccacatga agcagcacga cttcttcaag tccgccatgc ccgaaggcta cgtccaggag	900
cgcaccatct tcttcaagga cgacggcaac tacaagacc gcgccgaggt gaagttcgag	960
ggcgacaccc tgggtgaaccg catcgagctg aagggcacg acttcaagga ggacggcaac	1020
atcctggggc acaagctgga gtacaactac aacagccaca acgtctatat catggccgac	1080
aagcagaaga acggcatcaa ggtgaacttc aagatccgcc acaacatcga ggacggcagc	1140
gtgcagctcg ccgaccacta ccagcagaac acccccatcg gcgacggccc cgtgctgctg	1200
cccgacaacc actacctgag caccagtcg gccctgagca aagaccccaa cgagaagcgc	1260
gatcacatgg tcctgctgga gttcgtgacc gccgcccggg tcaactctcg catggacgag	1320
ctgtacaagt ccggactcag atctcgagct caagcttcga attctgcagt cgacggtacc	1380
gcgggcccgg gatccaccgg atctagataa ctgatcataa tcagccatac cacatttgta	1440
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aatgcaattg ttgttgtaa cttgtttatt gcagcttata atggtttaca ataaagcaat	1560
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tccactatta	aagaacgtgg	actccaacgt	caaagggcga	aaaaccgtct	atcagggcga	1860
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ggatcttctt	gagatccttt	ttttctgcgc	gtaatctgct	gcttgcaaac	aaaaaaacca	4080
cgcgtaccag	cgggtggttg	tttgccggat	caagagctac	caactctttt	tccgaaggta	4140

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actggcttca gcagagcgca gataccaaat actgtccttc tagtgtagcc gtagttaggc 4200
caccacttca agaactctgt agcaccgcct acatacctcg ctctgctaata cctgttacca 4260
gtggctgctg ccagtggcga taagtcgtgt cttaccgggt tggactcaag acgatagtta 4320
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cgaacgacct acaccgaact gagataccta cagcgtgagc tatgagaaa cgccacgctt 4440
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gccagcaacg cggccttttt acggttctcg gccttttctg ggccttttgc tcacatgttc 4680
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<210> SEQ ID NO 29
<211> LENGTH: 108
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequenec
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

```

```

<400> SEQUENCE: 29

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ctgtacaagt ccggactcag atctcgagct caagcttcga attctgcagt cgacgggtacc 60
cggggccccg gatccaccgg atctagataa ctgatcataa tcagccat 108

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```

<210> SEQ ID NO 30
<211> LENGTH: 8
<212> TYPE: PRT
<213> ORGANISM: Artificial
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

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<400> SEQUENCE: 30

```

```

Asp Tyr Lys Asp Asp Asp Asp Lys
1          5

```

```

<210> SEQ ID NO 31
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

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```

<400> SEQUENCE: 31

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```

Lys Pro Pro Thr Pro Pro Pro Glu Pro Glu Thr
1          5          10

```

```

<210> SEQ ID NO 32
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic Construct

```

```

<400> SEQUENCE: 32

```

```

Tyr Pro Tyr Asp Val Pro Asp Tyr Ala
1          5

```

```

<210> SEQ ID NO 33
<211> LENGTH: 5427
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Synthetic Construct

<400> SEQUENCE: 33

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cgagcaaaat ttaagctaca acaaggcaag gcttgaccga caattgcatg aagaatctgc	180
ttagggttag gcgttttgcg ctgcttcgcg atgtacgggc cagatatacg cgttgacatt	240
gattattgac tagttattaa tagtaatcaa ttacggggtc attagtctcat agcccatata	300
tggagtccg cgttacataa cttacggtaa atggcccgcg tggctgaccg cccaacgacc	360
cccgcccatt gacgtcaata atgacgtatg ttcccatagt aacgccaata gggactttcc	420
attgacgtca atgggtggag tatttacggt aaactgccc cttggcagta catcaagtgt	480
atcatatgcc aagtacgccc cctattgacg tcaatgacgg taaatggccc gcctggcatt	540
atgccagta catgacctta tgggactttc ctacttgga gtacatctac gtattagtca	600
tcgctattac catggtgatg cggttttggc agtacatcaa tggcggtgga tagcggtttg	660
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gtaggcgtgt acggtgggag gtctatataa gcagagctct ctggctaact agagaacca	840
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gtttaaacg gcctctaga ctcgagcggc cgccactgtg ctggatatct gcagaattcc	960
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cctcgactgt gccttctagt tgcagcccat ctgttggttg cccctcccc gtgccttcc	1080
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tgaatactca tactcttctt ttttcaatat tattgaagca tttatcaggg ttattgtctc 5340
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<210> SEQ ID NO 34
<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PARG RNAi

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<400> SEQUENCE: 34

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ccaguuggau ggacacuaau u 21

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```

<210> SEQ ID NO 35
<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PARG RNAi

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<400> SEQUENCE: 35

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```

uuacgaaggu accauagaau u 21

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<210> SEQ ID NO 36
<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ARH3 RNAi

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<400> SEQUENCE: 36

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ggacagaagc cuuguacuau u 21

```

```

<210> SEQ ID NO 37
<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ARH3 RNAi

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<400> SEQUENCE: 37

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```

ccauugcugg ugccuacuau u 21

```

```

<210> SEQ ID NO 38
<211> LENGTH: 21

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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Vector-target RNAi

<400> SEQUENCE: 38

guuuucacuc cagcuaacac a                               21

<210> SEQ ID NO 39
<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Vector-target RNAi

<400> SEQUENCE: 39

uucaaaagug aggucgauug u                               21

<210> SEQ ID NO 40
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PARP13 RNAi

<400> SEQUENCE: 40

gcucacggaa cuaugagcug aguuu                           25

<210> SEQ ID NO 41
<211> LENGTH: 1671
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 41

gcagtcctgcg cgcggatggc cgcagcggcg atggcggcag cggcaggtgg aggggctggc   60
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gtgggctcct tctacgaggc ccacgacacc gtcgacctga cgtcagtcct gcgtcatgtc   180
cagagtctgg agccggaccc cggcacgcc cggagtgagc ggacagaagc cttgtactac   240
acagatgaca cagccatggc cagggccctg gtgcagtcct tgctagccaa ggaggccttt   300
gacgaggtgg acatgggtca cagatttgct caggagtaca agaaagaccc tgacaggggc   360
tatggtgctg gagtagtcac tgtcttcaag aagctcctga accccaaatg tcgcgatgtc   420
tttgagcctg cccggggccca gtttaacggg aaaggtcctc atggcaatgg aggtgccatg   480
cgggtggctg gcactcctcc ggcctatagc agtgtccagg atgtgcagaa gtttgcccgg   540
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ctgggccaca tggaggatct ggagggtgat gccagtcctg tcttggtatg caggaggttg   720
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gacaccattg ccaccatggc tggggccatt gctggtgcct actatgggat ggatcaggtg  1020
ccagagagct ggcagcaaag ctgtgaaggc tacgaggaga cagacatcct ggcccaaagc  1080
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ttggaggggt acttgtggca ttttcttgta ttgtcttgga catgggatgt ggggagggtg	1440
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catcctagca ggggtcccca gcagcagggt gtgtggatga agggacaggc acttgcaccc	1560
agctgatcta ggtcacacct ggctcttggc tgccatgtgg cttattaaca gcttccagt	1620
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<210> SEQ ID NO 42

<211> LENGTH: 4276

<212> TYPE: DNA

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 42

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caattgcaga agcaggcagc ggcagagagg gaatggtgca ggcaggcgct gagaaggacg	180
cgcagtccat ctctctcagg ttagtgaaat gaggtctctc gcccggggcc ggcccgggga	240
cagtgcgctg ctggtccag catgaatgcg ggccccggct gtgaaccctg caccaagcga	300
ccccgctggg gcgcgctac aacttcgccg gctgcttcgg acgcccggag ctttcccagc	360
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tcgccagcct gcgtcccagg gcggggcggga cagcacagag gcagcgccac ctgcttggt	480
ttcaaacaaa agactattac cagttggatg gacactaaag gaatcaagac agcggaatca	540
gaaagtttgg atagtaaaaga aaacaacaat acaagaatag aatccatgat gatttctgta	600
caaaaagata acttttaacca acataatgta gaaaaattag aaaatgtttc tcagctaagt	660
cttgataagt caccactga aaaaagtaca cagtatttga accagcatca gactgcagca	720
atgtgtaagt ggcaaaatga agggaaacac acggagcagc ttttggaag tgaacctcaa	780
acagtaacc tggtaccaga gcagttagt aatgctaaca ttgatcggtc acctcaaat	840
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ttcatgagac tgcccaaac agaggacaga agaaaagaac agtgggaaac caaacatcaa	1560
agaacagaaa ggaagatccc taaatacgtt ccacctcacc tttctccaga taagaagtgg	1620

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aaaaaaaaaa aaaaaa 4276

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<210> SEQ ID NO 43
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PARP16 RNAi

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<400> SEQUENCE: 43

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cccaaguacu ucguggucac caaua 25

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<210> SEQ ID NO 44
<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PARP1 RNAi

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<400> SEQUENCE: 44

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aagccuccgc uccugaacaa u 21

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<210> SEQ ID NO 45
<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PARP2A RNAi

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<400> SEQUENCE: 45

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aaucagugua augaacuacu a 21

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<210> SEQ ID NO 46
<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PARP2B RNAi

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<400> SEQUENCE: 46

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aaugauucag cuauuagaag a 21

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<210> SEQ ID NO 47
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PARP3 RNAi

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<400> SEQUENCE: 47

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ggacccaggu guaugaggac uacaa 25

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<210> SEQ ID NO 48
<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PARP4 RNAi

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<400> SEQUENCE: 48

aaacaaggau uucuacuaag a

21

<210> SEQ ID NO 49

<211> LENGTH: 21

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: PARP5A RNAi

<400> SEQUENCE: 49

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21

<210> SEQ ID NO 50

<211> LENGTH: 21

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: PARP5B RNAi

<400> SEQUENCE: 50

aagcuucaga auggugcaaa u

21

<210> SEQ ID NO 51

<211> LENGTH: 25

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: PARP6 RNAi

<400> SEQUENCE: 51

cccaacaaug gaaacaucug agcaa

25

<210> SEQ ID NO 52

<211> LENGTH: 25

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: PARP6 RNAi

<400> SEQUENCE: 52

uugcucagau guuuccauug uuggg

25

<210> SEQ ID NO 53

<211> LENGTH: 25

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: PARP6 RNAi

<400> SEQUENCE: 53

gguucaaggc aagugguacc aucaa

25

<210> SEQ ID NO 54

<211> LENGTH: 25

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: PARP6 RNAi

<400> SEQUENCE: 54

uugaugguac cacuugccuu gaacc

25

<210> SEQ ID NO 55

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<211> LENGTH: 25
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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PARP6 RNAi

<400> SEQUENCE: 55

caaaguggaa guguuuggu acccu 25

<210> SEQ ID NO 56
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PARP6 RNAi

<400> SEQUENCE: 56

agggguagcca aacacuucca cuuug 25

<210> SEQ ID NO 57
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PARP6 RNAi

<400> SEQUENCE: 57

cagaacagag gauuccaaca uugaa 25

<210> SEQ ID NO 58
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PARP6 RNAi

<400> SEQUENCE: 58

uucaauguug gaauccucug uucug 25

<210> SEQ ID NO 59
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PARP7 RNAi

<400> SEQUENCE: 59

ugaggucuuu gaggccaaaua uuaaa 25

<210> SEQ ID NO 60
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PARP7 RNAi

<400> SEQUENCE: 60

uuuaauauug gccucaaaga ccuca 25

<210> SEQ ID NO 61
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
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<223> OTHER INFORMATION: PARP7 RNAi

<400> SEQUENCE: 61

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gacuuucugc aaggcacuug uauuu 25

<210> SEQ ID NO 62
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<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PARP7 RNAi

<400> SEQUENCE: 62

aaauacaagu gccuugcaga aaguc 25

<210> SEQ ID NO 63
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PARP7 RNAi

<400> SEQUENCE: 63

uccuccaccu cuugaagcaa cuuca 25

<210> SEQ ID NO 64
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PARP7 RNAi

<400> SEQUENCE: 64

ugaaguugcu ucaagaggug gagga 25

<210> SEQ ID NO 65
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PARP7 RNAi

<400> SEQUENCE: 65

aaugaugacc agaguuaccc uuauu 25

<210> SEQ ID NO 66
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PARP7 RNAi

<400> SEQUENCE: 66

aaauagggua acucugguca ucauu 25

<210> SEQ ID NO 67
<211> LENGTH: 25
<212> TYPE: RNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: PARP8 RNAi

<400> SEQUENCE: 67

ggaagauucu gaaggugaca augau 25

<210> SEQ ID NO 68
<211> LENGTH: 25
<212> TYPE: RNA

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<213> ORGANISM: Artificial Sequence
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 <223> OTHER INFORMATION: PARP8 RNAi

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<400> SEQUENCE: 82

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<210> SEQ ID NO 92
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<400> SEQUENCE: 92

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<400> SEQUENCE: 93

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<210> SEQ ID NO 95

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<400> SEQUENCE: 98

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<212> TYPE: RNA

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<220> FEATURE:

<223> OTHER INFORMATION: PARP12 RNAi

<400> SEQUENCE: 99

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<220> FEATURE:

<223> OTHER INFORMATION: PARP12 RNAi

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<212> TYPE: RNA
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<210> SEQ ID NO 106
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<400> SEQUENCE: 106

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gcugacccaa gaguagcacu uguua 25

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<210> SEQ ID NO 109
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aucugcugca cauaccacug aggca 25

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 <210> SEQ ID NO 117
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 <212> TYPE: RNA
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 <400> SEQUENCE: 117

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 <210> SEQ ID NO 118
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 <212> TYPE: RNA
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 <210> SEQ ID NO 120
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<210> SEQ ID NO 121
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<220> FEATURE:
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<211> LENGTH: 25
<212> TYPE: RNA
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<400> SEQUENCE: 123

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<210> SEQ ID NO 124
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<212> TYPE: RNA
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<220> FEATURE:
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<400> SEQUENCE: 124

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<210> SEQ ID NO 125
<211> LENGTH: 25
<212> TYPE: RNA
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<220> FEATURE:
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<210> SEQ ID NO 126
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<220> FEATURE:
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<210> SEQ ID NO 127
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<212> TYPE: RNA
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<210> SEQ ID NO 128

<211> LENGTH: 25

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<223> OTHER INFORMATION: PARP15 RNAi

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<212> TYPE: RNA

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<220> FEATURE:

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<212> TYPE: RNA

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<220> FEATURE:

<223> OTHER INFORMATION: PARP16 RNAi

<400> SEQUENCE: 130

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<211> LENGTH: 25

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<220> FEATURE:

<223> OTHER INFORMATION: PARP16 RNAi

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<220> FEATURE:

<223> OTHER INFORMATION: PARP16 RNAi

<400> SEQUENCE: 132

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<210> SEQ ID NO 133

<211> LENGTH: 25

<212> TYPE: RNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: PARP16 RNAi

<400> SEQUENCE: 133

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<210> SEQ ID NO 134

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<211> LENGTH: 25
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 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
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<400> SEQUENCE: 134

gacuugagcc ugcccucac auaca

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<210> SEQ ID NO 135
 <211> LENGTH: 25
 <212> TYPE: RNA
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 <220> FEATURE:
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<210> SEQ ID NO 136
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 <223> OTHER INFORMATION: PARP16 RNAi

<400> SEQUENCE: 136

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25

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The invention claimed is:

1. A method of inhibiting stress granule formation in a subject comprising administering to said subject a therapeutically effective amount of one or more poly-ADP-ribose polymerase (PARP)12 inhibitor(s) and/or one or more PARP13 inhibitor(s) wherein:

(a) said one or more PARP12 inhibitor(s) is (i) an antibody or an antibody fragment that selectively binds PARP12, or (ii) an RNA aptamer comprising the sequence of any one of SEQ ID NOs: 99-106; and/or

(b) said one or more PARP13 inhibitor(s) is (i) an antibody or an antibody fragment that selectively binds PARP13.1 and/or PARP13.2, or (ii) an RNA aptamer comprising the sequence of any one of SEQ ID NOs: 40 and 107-113, and

wherein said administering results in the inhibition of stress granule formation in said subject.

2. The method of claim 1, wherein:

(a) said one or more PARP12 inhibitor(s) selectively decreases the expression and/or one or more activities of PARP12, and/or

(b) said one or more PARP13 inhibitor(s) selectively decreases the expression and/or one or more activities of PARP13 isoform 1 (PARP13.1) or PARP13 isoform 2 (PARP13.2).

3. The method of claim 2, wherein:

(a) said decrease in expression is a decrease in the level of one or more nucleic acid(s) comprising a nucleic acid sequence having at least 95% sequence identity to PARP12 (SEQ ID NO: 18), PARP13.1 (SEQ ID NO: 19), or PARP13.2 (SEQ ID NO: 20), or in the level of one or more polypeptide(s) encoded by said one or more nucleic acid(s); or

(b) said one or more activities of PARP12, PARP13.1, or PARP13.2 is (i) poly-ADP-ribosylation of a target protein localized in a stress granule or a target protein involved in the formation or disassembly of a stress granule, or (ii) the formation or nucleation of a stress granule.

4. The method of claim 1, wherein said subject has, or is at significant risk of developing, a stress granule-related disorder.

5. The method of claim 4, wherein said stress granule-related disorder is a cardiovascular disorder, an inflammatory disorder, a neurological disorder, or an ischemic reperfusion injury.

6. The method of claim 5, wherein:

(a) said cardiovascular disorder is selected from the group consisting of an aneurysm, angina, atherosclerosis, stroke, cerebrovascular disease, congestive heart failure, coronary artery disease, myocardial disease, peripheral vascular disease, granulomatous myocarditis, chronic myocarditis, myocardial infarction, and primary hypertrophic cardiomyopathy;

(b) said inflammatory disorder is selected from the group consisting of autoimmune diseases, asthma, allergic intraocular inflammatory diseases, arthritis, atopic dermatitis, atopic eczema, cirrhosis, Crohn's disease, ulcerative colitis, diabetes, hemolytic anemia, inflammatory dermatosis, inflammatory bowel disorder, systemic lupus erythematosus, psoriasis, rheumatoid arthritis, Wegener's granulomatosis, Hashimoto's thyroiditis, chronic pancreatitis, and reactive lymphoid hyperplasia; or

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(c) said neurological disorder is selected from the group consisting of multiple sclerosis, Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, retinosa pigmentosum, macular degeneration, traumatic brain injury, stroke, and peripheral 5 neuropathy.

7. The method of claim 1, wherein said RNA aptamer consists of the sequence of any one of SEQ ID NOs: 99-106.

8. The method of claim 1, wherein said RNA aptamer consists of the sequence of any one of SEQ ID NOs: 40 and 10 107-113.

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